THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME I JULY, 1925 NUMBER 4

STUDIES ON ENDOTHELIAL REACTIONS

IX. THE FORMATION OF RETICULUM IN THE LESIONS OF EXPERIMENTAL TUBERCULOSIS IN RABBITS *

NATHAN CHANDLER FOOT, M.D.

(From the Department of Pathology, University of Cincinnati College of Medicine, and Cincinnati General Hospital, Cincinnati)

Introduction. Investigations on the nature of the reticulum in the lesions of tuberculosis have recently been given impetus by the studies of Miller (1923) and Long and Miller (1924). In the past, Mall's (1891, 1896) work on the normal reticulum was not followed up with any marked diligence until Maresch modified the Bielschowsky technic of silver impregnation to apply to it, whereupon his much less complicated method stimulated a series of investigations by Rössle and his pupils, Kon (1908), Russakoff (1909), and Yoshida (1909) — all of whom studied the reticulum of various organs under normal and pathological conditions. Their conclusions were that this form of fibrous tissue is a direct product of cellular activity, that it may present true reticular structure, or may be a reticular thickening in sheet-like membranes (particularly in epithelial organs), and that it probably represents a precollagenous form of fibrous connective tissue. Neuber (1912) subsequently made a study of the cardiac reticulum under normal and pathological conditions. All these investigators agree that this material is entirely independent of the elastic fibrous tissue, and present convincing proof of this assertion. Russakoff made a painstaking investigation of the reticulum in pulmonary tubercles, and Miller has supplemented his work with an equally meticulous study of the reticulum in the tubercles in a child's lung. He agrees that elastic tissue and

* Received for publication March 19, 1925.

reticulum are unrelated, and affirms his belief that the latter presents an early stage in the formation of collagenous connective tissue, under certain circumstances.

The histogenesis of reticulin fibrils is still very poorly understood; the earlier writers believe it to be formed within endothelial cell processes, and Kon, studying its production in human embryos, states that it is laid down in the fourth month of development as granular linear structures in these processes, the granules becoming fused to form threads in the fifth or sixth month. Miller takes exception to this theory, pointing out that the fibers form so complicated and abundant a network that their formation could scarcely be explained in this manner. That there is a definite relationship between cellular activity and fibril production seems to be agreed; Corner (1920) has stressed the almost universal association of endothelial cells and reticulin fibrils and concludes that the latter are usually produced by the capillary endothelium; the association of the reticuloendothelial cells with reticulum formation has been known since Mall's first paper; and it seems that the fibroblast may also be closely associated with reticulum, particularly in the medullary rays of the kidney. Whether the reticulin forming these fibrils differs from that laid down near endothelial cells remains to be seen. No reticulum is seen in the glomerular tuft under ordinary circumstances, although I have occasionally seen it in rabbit kidneys.

White and Smith (1924) have recently suggested that there is a relationship between fibrin formation and the production of reticulin, that the former might be converted into the latter by the agency of the endothelium. They point out that recent investigation has proved that reticulin fibrils are formed outside of cells, appearing in the embryonal body fluid before cells of any sort are demonstrable in their proximity. They note that both Hertzler and Baitsell (1915, 1916) have shown that fibrin may be converted into connective tissue fibrils "which cannot be distinguished, either in their structure or tinctorial reactions, from the fibrils of reticulum and white fibrous tissue." They apply these theories to the formation of tubercle reticulum, believing that this may be produced by the fibrin at the center of the tubercle. They state that reticulin is probably a gelatinous material until fixed by the various coagulant reagents used for this purpose, when it takes on the familiar thread-like form, an hypothesis also advanced by Rössle and Yoshida.

The foregoing review naturally leads to the question: What is reticulin? Is it purely an artefact, or does it exist as a gelatinoid network before fixation? The term "reticulin" is applied to the substance forming the fibrillar network which is called "reticulum." Is every fibrillar substance that takes the Bielschowsky silver impregnation (exclusive of nervous tissue) composed of reticulin? That it is not an artefact is indicated by the experiment of Rössle and Yoshida, who bruised and pinched freshly removed lymphnodes before fixation and later found the reticulum correspondingly distorted and compacted into a felt-like material in the sections cut after fixation. Whether or not the silver impregnation is exactly specific for reticulin we cannot say, but it would appear to be so.

Thus we are faced by several questions: Does the tubercle reticulum represent a direct transformation of fibrin into reticulum? Is the agency of cells necessary for its production? Is it laid down within those cells as Russakoff claimed, or outside of them as Miller, and White and Smith suggest? Does reticulin become further elaborated into collagen? To attempt an answer to these questions, tubercles were produced in rabbits, representing lesions in various stages of the progressive development of the process, and sections from these were stained specifically for fibrin, reticulum, and collagen.

Experiments. Seven rabbits were inoculated intravenously with 0.2 mg. per kgm. body weight of a bovine strain used in the experiments described in the preceding paper of this series. (Foot, 1923.) One rabbit was killed after one week, one died of intercurrent infection at eleven days, and tuberculosis killed one at fourteen, two at sixteen, and one at eighteen days, thus affording a graded scale of lesions. Two rabbits from another experiment were added to this series. They had both received the same dose of the same strain intravenously eighteen days before death.

A second series, composed of older lesions, was selected from material collected by Dr. Austin, head of this department, who kindly placed it at the writer's disposal. In this set, stages were obtained representing five weeks (1), six weeks (2), eight, nine, fifteen, seventeen, and twenty-three weeks (1 each). These were all produced by the subcutaneous inoculation of 5 mg. per kgm. of the same bovine strain.

Technic: The tissue was fixed in Zenker's fluid, imbedded in paraffin and cut to five microns; in each case, sections were made from the lung, liver, spleen, and kidney. For the study of fibrin, Mallory's phosphotungstic acid hematoxylin and the Gram-Weigert stain were used; for that of collagen, Weigert's modification of the Van Gieson stain and the phosphotungstic acid hematoxylin technic; and for that of the reticulin, the writer's modification of the Bielschowsky-Maresch silver impregnation. (Foot, 1924.) Harris' hematoxylin was substi-

tuted for Weigert's iron-hematoxylin in the latter and the results were very much improved, owing to the greater range and variety of color.

By means of this combination of methods the production of fibrin could be detected and studied; collagen fibers could be distinguished as soon as they appeared; and reticulin and collagen fibrils could be demonstrated and distinguished one from the other in the silver impregnations. Sets of routine hematoxylin-eosin sections were prepared in each case, as a check on the general topography of the lesions and tissues; in all instances the sections were practically serial.

Fibrin and Reticulin: That there is no immediate or direct transformation of fibrin into reticulin is readily ascertained in this experiment.

No fibrin is found in the seven-day tubercles, although it is occasionally present in vascular clots. The eleven-day rabbit died of fibrinous pneumonia, pleuritis, and pericarditis; the fibrin stains well in the exudate, and some is found in the pulmonary and hepatic tubercles that have developed as a result of the specific inoculation, but it is centrally located and quite unlike reticulin in its appearance and tinctorial reaction. In the twelve-, fourteen-, and one of the sixteen-day rabbits, fibrin is found in small quantities in the liver tubercles, usually centrally; it is very variable in the pulmonary tubercles, appearing in small quantities in the lumina of capillaries at the periphery of the lesions in one case only — the two-week rabbit. In the more advanced lesions of the second series of subcutaneously inoculated rabbits it is occasionally present, but inconstantly and in no relation to the production of reticulin or collagen.

The fibrin network has not the faintest resemblance to reticulum; it is coarser, more translucent, its threads run a far straighter course and have nodal thickenings at the intersections, and it is usually found at the center, rather than at the periphery of the tubercles. Comparing serial sections, one stained for fibrin, the other for reticulin, one finds that there is no similarity in appearance, or in distribution, between the fibrinous network and the reticulum. The one is coarse, central, and straight; the other fine, peripheral, and curly or waving. The fibrin might stain faintly with acid fuchsin in the Van Gieson stain, but it is never impregnated by the silver. For these reasons it would seem almost certain that there is no direct relationship between the two and no immediate transformation from one to the other. Furthermore, there is a well-developed reticulum in the pulmonary tubercle at one week, although no fibrin is demonstrable. Later lesions show the same thing, the production of fibrin and of reticulin appear to be independent processes.

Reticulin and Collagen (First Series of Rabbits). In the first series of lesions it is readily ascertained that the reticulum is best studied in the liver tubercles, where it does not appear in quantity until about the fourteenth day. In pulmonary tubercles there is already a delicate reticulum at the end of a week. In the spleen the investiga-

tion is very difficult and unsatisfactory, on account of postmortem autolysis, the presence of splenic reticulum, and peculiar precipitates in the pulp. The kidney is already too rich in reticulum and the lesions too scanty to render its study very profitable.

Liver. The liver tubercles first show a very delicate reticulum after eleven days have elapsed. This seems to be continuous with the sinusoidal reticulum, which is very scanty in rabbits as compared with that of human livers. The fibrils are often beaded and they are branching and short. They are most definite at the periphery of the tubercle and more plentiful in lesions occurring near Glisson's capsule than in those at some distance therefrom. It is impossible to say that these fibrils are produced within cells; they lie at the periphery of endothelial phagocytes and are usually continuous with neighboring sinusoidal fibrils. At first they are more delicate in the tubercles than in the sinusoids, but after two weeks they become so coarse and prominent that one may readily locate tubercles by looking for dark spots in the field. The sinusoidal reticulum becomes strikingly thicker and more prominent in the neighborhood of the tubercles. It has just been stated that reticulin fibrils do not run within the cytoplasm of endothelial cells, but there is one exception: they can be demonstrated penetrating that of the giant-cells and running through them between the nuclei. This could be explained by the assumption that these syncytia are formed by the fusion of endothelial phagocytes which had formed fibrils at their periphery prior to fusing, which would include them in the common cytoplasm.

The gradual transformation of reticulin into collagen is strongly indicated in all the tubercles of this first series; the older the tubercle, the pinker become the coarser fibrils. As the silver impregnation colors collagen old-rose, and the superimposed Van Gieson stain renders them scarlet, this change is easily followed.

Summarizing this first series of lesions, we find that reticulum is formed before either fibrin or collagen; that there is no evident relationship between fibrin and reticulin, whereas there is a striking parallelism between the formation of reticulin and collagen; and that the process is best studied in its inception in the hepatic tubercles produced by intravenous injection of tubercle bacilli. We also see that a definite causal relationship between cells and reticulum cannot be established by this method of procedure, although the universal presence of endothelium in the neighborhood of the reticulum strongly indicates that the former plays some part in the production of the latter.

Second Series of Rabbits. The series of older lesions, produced by subcutaneous inoculation and ranging from five to twenty-two weeks between inoculation and death, present several features differing from those of the first series. The exact age of the systemic

tubercles is necessarily indefinite, as they result from infection from the primary subcutaneous tubercles; the subcutaneous tubercles may bring about allergic phenomena which could influence reticulum formation (see Long and Miller, 1924); and the development of the systemic tubercles is more difficult to follow stage by stage. The following facts are noted: the liver ceases to be the best organ in which to study reticulum formation, as this is not only very variable, but many of the hepatic tubercles show no reticulum whatsoever. The spleen continues to be poor terrain for observation, and the kidney varies according to whether it be infected or not — if tubercles are produced, there is a great deal of dense reticulum formed, more than in the other organs. The most instructive organ in this series is, therefore, the lung; in considering it, it will be best to review the results of observation on both series of rabbits, commencing at one week and continuing stage by stage through the twenty-two week rabbit.

Lung. There are, as we know, two types of pulmonary lesion in this disease: one starting within the alveolar wall, and one in which there is intraalveolar exudate. The progress of the two lesions may be traced along one or the other of these two lines, or they may be found to be combined, which is usual. In the first case one notes a local multiplication of endothelial cells near the alveolar capillary; there is a nodular thickening composed of ovoid epithelioid cells. A delicate reticulum forms immediately and its fibrils are very intimately connected with the endothelial cells, but they are directly continuous with the preëxisting pericapillary reticulum and appear to be prolongations of its fibrils rather than independently formed products of cellular activity, or cytoplasmic processes (Fig. 1). This growth continues and the tubercle bulges into the neighboring alveolus, or alveoli (Fig. 2). At the same time, an exudate of endothelial phagocytes appears in the air-space of the alveolus and if the epithelial lining remain in tact no reticulum is seen among the cells of this exudate; if the epithelium become necrotic, however, the tubercles break into the air-spaces and grow there, carrying their reticulum with them and sending branches thereof among the cells of the exudate (Figs. 3 & 5). There is no indication that these cells produce reticulum that is independent of, and unconnected with, that of the alveolar wall. The latter becomes destroyed, its capillaries disappear, and the alveolar boundaries are obliterated. If caseation supervene, the reticulum is largely destroyed (after a short period of brisk proliferation) and remains as short, thick, rod-like masses in the caseous material. The reticulum of the alveolar walls bounding such caseous areas becomes greatly increased and thickened in situ (Fig. 4). There is a variant of this process: near veins it is noted that there is little thickening of the alveolar wall, but an exudate of discrete endothelial phagocytes fills each alveolar sac. Reticulum appears to run directly from the wall of the vein into the exudate and to ramify among its cells, without awaiting the destruction of the alveolar wall. The latter process follows eventually, and the vessel becomes surrounded by a sheath-like mass of

epithelioid tissue, which is liberally supplied with reticulum. If the vein be the site of a tuberculous thrombus (as often happens in this experiment) the reticulum runs into this thrombus, as well as out into the perivascular tissue (Figs. 6 & 7). Either of these processes may result in the complete wiping-out of the pulmonary topography over considerable areas and the laying down of extensive fields of reticulum (Fig. 8).

The invasion of the intraalveolar exudate may become very extensive (Fig. 5) so that the alveolar boundaries are scarcely recognizable, having lost their capillaries and epithelium and being interspersed by fields of reticulated exudate. After eighteen days of development, one notices that the coarser reticulum begins to take on a reddish color and this increases steadily in its intensity until many of the fibers are quite red, indicating that they have been converted into collagen. In the older lesions, at about fifteen weeks, one finds that the formless fields of reticulum are becoming converted into networks of rather dense, chiefly collagenous fibers, and that the interstices are filled with lymphocytes, plasma cells, and endothelial phagocytes, all of which are separate from one another and from the fibrils of the matrix. In other words, a typical scar has been produced, although this interpretation of its production differs from the usual conception of fibroblastic proliferation, which is notably absent. This idea implies destruction, collapse and shrinkage, and proliferation of the preëxisting reticulum with conversion of the latter into collagen, rather than invasion by fibroblasts and formation of fibrous tissue through their agency.

The first complete scars noted in this series are seen in the lung of the seventeen-week rabbit (Fig. 9). They are composed of loose complexes of thick, collagenous fibers, loosely scattered cells of various types, and vessels of moderate caliber and thin walls, some of them more or less completely thrombosed by tuberculous tissue which is well preserved, provided with a reticulum, and not necrotic. The coarse collagen fibers are continuous with finer reticulin fibers, and these with a delicate reticulum of interlacing fibrils. (Cf. Miller, 1924.) Transitions of reticulin to collagen are observed all through these fields; a given fiber may be red at one end and black at the other, or blackish nodes of reticulin may be found imbedded in red collagen fibers. Fig. 9 represents this condition, the use of red light for the photography renders the collagen gray, while the reticulum stands out as black lines, dots, or dashes on this gray background. This may give one a clue as to the steps in the process of transformation from reticulin to collagen. Although there is a good deal of collagen present after eighteen days, it is not conspicuous in the silver sections until a month or more has elapsed, and then the transformation is incomplete, much reticulum remaining unconverted. Only the silver impregnation can demonstrate this satisfactorily (Fig. 10).

A study of the reticulum of tubercles in organs other than the lung, in the second series, adds little or nothing to the impressions already gained from the first.

Discussion. The results of this experiment throw considerable light upon the formation of reticulum in tubercles, but they have not cleared up the mystery of its histogenesis to the extent one might have anticipated. It will be necessary to experiment with

the formation of reticulin in tissue cultures, in connection with the mononuclears of the blood, along the lines taken by Margaret Lewis in her recent brilliant experiments (Lewis, 1925), before we can say just what rôle the endothelium plays in the formation of this substance. Such an experiment will form the basis for a subsequent paper. It can be said, however, that one gains the distinct impression, while examining the slides of this series, that the new reticulum is merely a prolongation of the old; that it represents a growth by accretion, as it were, rather than a laying down of reticulin in cell processes. It may be, and probably is the result of secretions of fibroblasts or of cellular derivatives of the vascular or reticulo-endothelium: a process analogous to the precipitation of a fibrin network through the activity of fibrinogen, which is now known to be contained in largest quantities in those organs that are richest in endothelium. That there is no direct transformation of fibrin into reticulin is sufficiently indicated in the preceding text. The assertions of earlier writers as to the conversion of reticulin into collagen is amply borne out by these experiments; it would seem that there can be little doubt as to the truth of their beliefs.

Conclusions

1. The histogenesis of the reticulum is not satisfactorily solved by this method of study; cellular derivatives of the endothelium seem to play an important part in forming reticulum, for they are always present in its vicinity — but they do not appear to produce it by intracellular activity.

2. It is indicated that newly formed reticulum is a product of pre-existing reticulum, as it is always continuous with it.

3. Fibrin plays no direct part in the production of reticulin.

4. Reticulin is apparently converted directly into collagen, but the manner in which this transformation is effected has yet to be explained.

5. Caseation and reticulin formation are incompatible; after a preliminary proliferation in areas of early caseation, the reticulum is destroyed.

6. The presence of tubercles in an organ appears to stimulate the growth and to cause a coarsening of the normal, preëxisting reticulum in their immediate vicinity. This might be interpreted as a forerunner of the production of newly-formed reticulin fibrils.

7. More reticulum is produced in those tubercles occurring in or near portions of an organ already rich in that tissue. Conversely, it is scanty in those occurring at some distance from the stroma of the organ.

8. The importance of collapse in the process of scar formation in chronic tuberculosis of the lung is emphasized in sections impregnated with silver.

REFERENCES

Baitsell, G. A. 1915. J. Exp. Med., xxi, 455.
Baitsell, G. A. 1915–16. Anat. Rec., x, 175.
Baitsell, G. A. 1916. J. Exp. Med., xxiii, 739.
Corner, W. G. 1920. Carnegie Institution of Washington, Contributions to Embryology, ix, 85.
Foot, N. C. 1923. J. Exp. Med., xxxviii, 263.
Foot, N. C. 1924. J. Lab. & Clin. Med., ix, 777.
Kon, Y. 1908. Arch. f. Entwicklungsmechanik. d. Organ., xxv, 492.
Lewis, M. 1925. Am. J. Path., i, 91.
Long, E. R., and Miller, W. S. 1924. Tr. 20th Ann. Meeting Nat. Tuberculosis Assn., 285.
Mall, F. P. 1891. Abhndl. d. math. phys. Classe, Kgl. sächs. Gesellsch. d. Wissensch., xvii, 299.
Mall, F. P. 1896. Johns Hopkins Hospital Rep., i, 171.
Miller, W. S. 1923. Am. Rev. Tuberculosis, vii, 141.
Neuber, E. 1912. Beitr. z. path. Anat. u. z. allg. Path., liv, 350.
Rössle, R., and Joschida (Yoshida), T. 1909. Beitr. z. path. Anat. u. z. allg. Path., xlv, 110.
Russakoff, A. 1908. Beitr. z. path. Anat. u. z. allg. Path., lxv, 476.
White, W. C., and Smith, M. I. 1924. Tr. Clin. & Climat. Assn.

DESCRIPTION OF PLATES LVI-LVII

PLATE LVI

Fig. 1. A tubercle at one week, bulging from either side of an interalveolar septum into the neighboring air-sacs. Thickening of adjacent alveolar wall. The reticulin fibrils are already abundant and there is thickening of the original reticulum. The newer fibers are continuous with the older.

Fig. 2. Larger tubercle at eleven days. Alveolar boundaries are beginning to be lost, there is great thickening of the preëxisting reticulum and a tendency for new fibrils to invade the intraalveolar exudate.

Fig. 3. An area which, in a hematoxylin-eosin section, would pass for a conglomerate tubercle. Note that the alveolar reticulum, although dispersed and indefinite, still maintains enough of its original topography to suggest about nine air-sacs. There is invasion of the exudate by newly-formed reticulum. Eighteen days after inoculation.

Fig. 4. Similar to Fig. 3, but shows the usual marked thickening of the original reticulum about a caseous area, as well as the formation of reticulum in the exudate. Twelve-day lesion.

Fig. 5. The alveolar boundaries are hard to make out, the walls thickened, the capillaries gone. The reticulum is more diffuse and branching and does not confine itself to the pericapillary tissue. Six-week lesion.

Fig. 6. A reticulated intravenous thrombus composed of epithelioid cells, eighteen days after inoculation. The continuity of the intravascular with the perivascular reticulum is afforded by the original reticular ring within the wall of the vein, from which the new reticulum seems to spring. The perivascular alveoli are invaded by a fibrous network of reticulin which is in far closer relationship to the original vascular reticulum than it is to the endothelial phagocytes, which seem rather to have become enmeshed in its fibrils than to have produced them.

Plate LVII

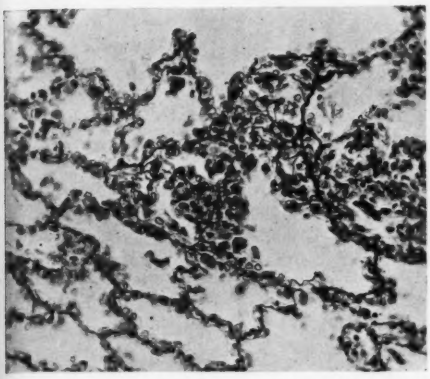
Fig. 7. Completed tuberculous thrombus in a large vein fifteen weeks after subcutaneous inoculation. Both intra- and perivascular reticula are very coarse.

Fig. 8. The earliest type of diffuse scarring by collapse of air-sacs, loss of topography, and diffuse proliferation of the reticulum. This is not an interlobular septum. Fourteen days after inoculation.

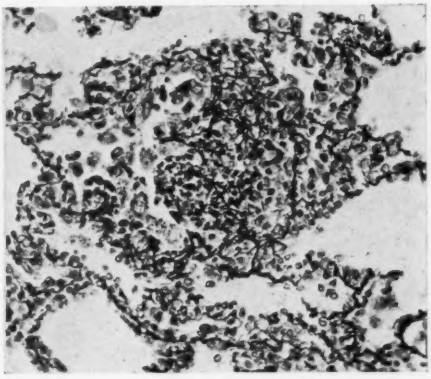
Fig. 9. Presumably a similar area, fifteen weeks after subcutaneous inoculation. Alveoli destroyed, vessels increased in size, much of the reticulin converted into collagen, which photographs gray. Note the nodal, or beaded appearance of the surviving reticulin, which is imbedded in collagen. This gives some clue as to the manner in which the latter replaces the former.

Fig. 10. A scar after seventeen weeks. It is mostly collagen, with comparatively little reticulin remaining. The alveoli are destroyed, or collapsed and converted into "acini." There are many lymphocytes and plasma cells lying enmeshed in the fibrous tissue.

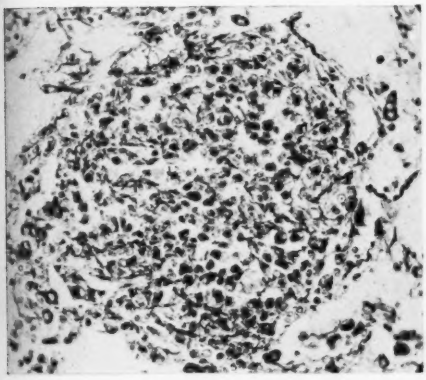
Note: All these photomicrographs show a magnification of 350 diameters; they are all taken from sections stained by my modification of the Bielschowsky-Maresch method; the exposure time is the same in every case; therefore they are strictly comparable with one another. They were all taken with red light, Wratten filter "F," in order to subdue the details of the cellular elements and emphasize the reticulin fibers, as well as to tone down the bright-red collagen fibrils so that they would photograph gray, instead of black. This differentiates them from the reticulum which stains deep black in these sections.



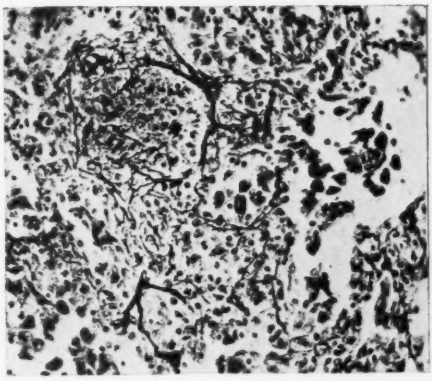
1



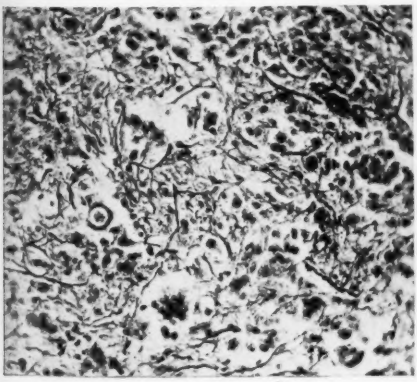
2



3



4

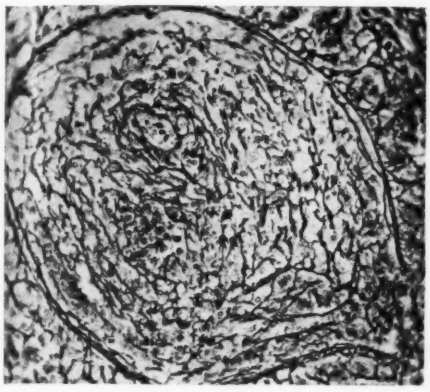


5

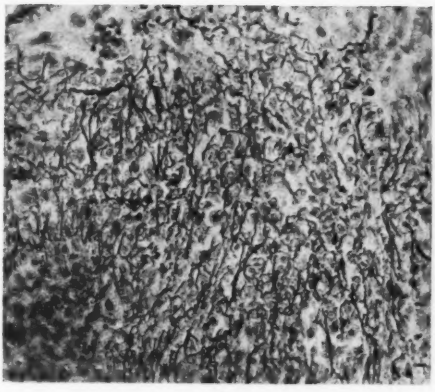
6

Foot

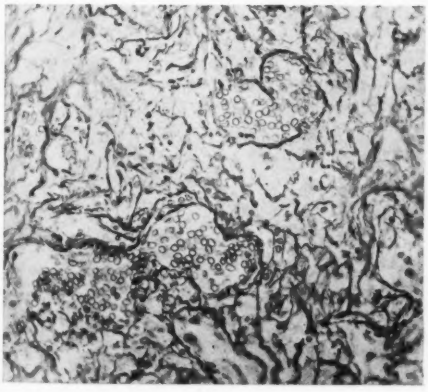
The formation of reticulum



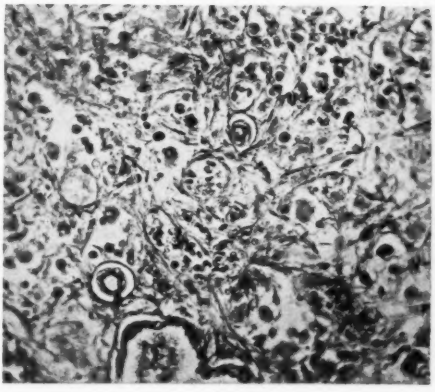
7



8



9



10

Foot

The formation of reticulum

THE STATE OF THE CARDIAC MUSCLE IN HYPERTROPHY AND ATROPHY *

HOWARD T. KARSNER, OTTO SAPHIR, and T. WINGATE TODD

(From the Departments of Pathology and Anatomy, School of Medicine, Western Reserve University, Cleveland, Ohio)

INTRODUCTION

It is generally considered that in hypertrophy of the heart there is increase in the size of some of the muscle fibers and in atrophy there is a decrease in size of many of the fibers, but this view is not sufficiently supported by accurate study. The recent literature shows a disposition to accept the hypothesis that in the human heart, hypertrophy is due almost entirely to an increase in the size of the fibers and that the converse is true in atrophy. The study here presented deals with this question on the basis of measurements of the transverse diameters of large numbers of fibers in the normal, hypertrophic and atrophic human heart. The hearts examined were selected so as to avoid confusion due to coincident disease of the myocardium. The nuclei were counted in many fields, but without regard to the number of fiber units (the part of the fiber between two intercalated discs), since it is admittedly difficult to be sure, in all instances, of the boundaries of these units. The study deals with large numbers of fibers so as to reduce the error of random sampling. The data obtained have been carefully reduced on a mathematical basis.

LITERATURE

Many of the older writers, including Vogl, Köllicker, Lebert, Hyrtl and Rokitansky, were of the opinion that in hypertrophy of the heart the muscle is the seat of a hyperplasia or multiplication of fibers rather than simple hypertrophy. As late as 1911 Wideroe appears to hold this view. Included among those who have held the view that there are both hyperplasia and hypertrophy are Wedl, Foerster, Friedrich, Paget, Rindfleisch, Zielonko, Orth and Adami. Kaufmann and Aschoff both express the view that the process is

* Received for publication April 24, 1925.

one of pure hypertrophy. These views are also supported by the admirable studies of Tangl and of Goldenberg.

The subject of atrophy in general has been carefully studied by Bradley and his collaborators, who regard the process as autolytic in character. Jamin, in an experimental and morphologic study of skeletal muscle, concludes that simple atrophy such as that due to inactivity is different from a degenerative atrophy in the absence of retrogressive changes other than the atrophy itself. In his concept the term atrophy implies a reduced functional capacity. He finds as the result of inactivity a reduction in size of the fibers. This is in conformity with the work of Durante and of Schmidtman, who, however, also find an occasional large or hypertrophic fiber. All these investigators have found an increase in the number of nuclei, a feature emphasized in most textbooks on pathology. There is still question, however, as to whether the information yielded by the study of skeletal muscle is applicable to cardiac muscle. As to the relations between nuclei and muscle, the work of Collier and of Edens is of especial importance. The former finds that in the hypertrophic heart the ratio between area of nucleus and area of cytoplasm is essentially the same as in the normal heart. Edens determined the ratio of purine and total nitrogen in cardiac muscle with the idea that the method will furnish an estimate of the ratio of nuclear and cytoplasmic substance. In the normal and hypertrophic heart the ratio is about the same but in the atrophic heart the purine nitrogen is significantly increased, thus indicating a relative increase of nuclear substance.

In our study an attempt was made, with the aid of Dr. N. W. Ingalls, to reconstruct the heart muscle fibers by serial section and wax plates. This was found to be impracticable and the method given below was adopted.

Method

Hearts were selected so as to be free from other conditions than those to be studied. Thus, the picture is not confused with coincident inflammatory or destructive changes. Age was regarded as of importance and the hearts were from patients about 30 years of age. The normal heart was from a patient 32 years of age, the hypertrophic heart from one 36 years of age, the atrophic heart from one 28 years of age. All were males and of medium stature. The hyper-

trophic heart was from a patient with chronic glomerulonephritis, the other two from patients with pulmonary tuberculosis. The organs were freed of adventitious tissue, weighed fresh and the volume determined by water displacement in the same manner in all three cases. Blocks were made from corresponding positions near the base of the left ventricle. This situation was selected because it gave the best picture of longitudinal sections of the fibers. The blocks were fixed in 4 per cent formaldehyde, embedded in paraffin, cut in serial sections 5 micra thick including the entire thickness of the heart wall, and stained with hematoxylin and eosin.

A filar micrometer ocular was used in the study. The size of the field selected, on the basis of convenience, was uniformly 0.15 by 0.2 millimeters as determined by the stage micrometer. In each field the number of longitudinally cut fibers was counted. In order to avoid duplication only those fibers parallel to the long axis of the marked field were included. Similarly, the number of nuclei was counted by fields rather than in reference to fiber units because of the difficulty of being certain of the position of the intercalated discs. For this purpose the 16 mm. objective was employed. Knowing the thickness of the section each field represents 0.00015 cubic millimeter. The transverse diameter of longitudinally cut fibers was measured by the graduated drum of the filar micrometer. Here the 4 mm. objective was used. Each 0.1 division of the drum corresponds to 3.5 micra, but in order to save labor the measurements are referred to in multiples of the drum divisions. In each field one large fiber and one small fiber were measured. As will be seen from the tables large numbers of fields were counted and large numbers of fibers measured.

Results

Number of fibers per field

	Maximum	Minimum	Average
Normal heart	19	7	11.5 ± 0.021
Atrophic heart	17	7	10.8 ± 0.023
Hypertrophic heart	11	5	7.4 ± 0.015

A comparison of the averages for the normal heart and hypertrophic heart shows an apparently significant difference, but between the normal and atrophic hearts the difference is not so great. An analysis of the figures is given in Table 1 and shown graphically

in Fig. 1. In the normal heart there is a fairly wide distribution of the various numbers per field with the maximum of fields in those which contain 12 fibers, close to the average of 11.5. In the hypertrophic heart the maximum of fields is in those which contain 8 fibers slightly above the average 7.4. There are no fields containing

TABLE 1

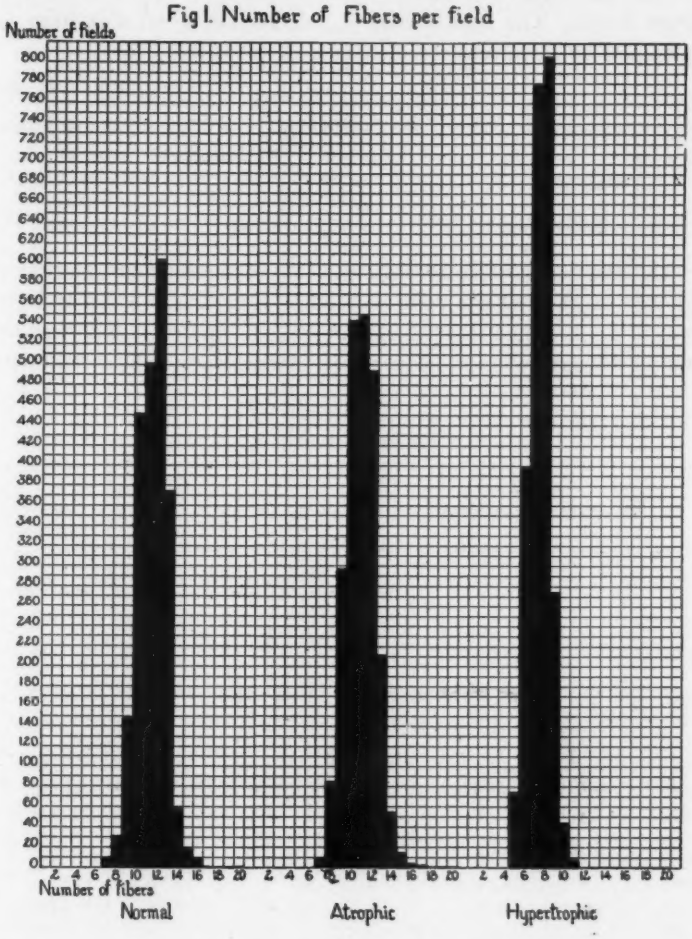
Number of fibers per field

Number of fibers	Number of fields		
	Normal	Atrophic	Hypertrophic
5	0	0	76
6	0	0	398
7	10	8	778
8	31	86	808
9	149	298	266
10	451	546	42
11	502	548	8
12	612	494	0
13	376	212	0
14	59	60	0
15	19	16	0
16	9	4	0
17	2	2	0
18	2	0	0
19	2	0	0
	2,224	2,274	2,376

the larger numbers of fibers. The small distribution of fields indicates an approach toward greater uniformity than is true of the normal heart. In the atrophic heart the curve differs so that although the maximum of fields is in those which contain 11 fibers (average 10.8), nevertheless, the top of the curve is more flat than in the normal. It rises to and falls from its summit more steeply than the curve of the normal heart and presents fewer fields containing more than 11 fibers. These facts point toward a disposition to greater uniformity than is true of the normal.

The probable error of an average is a figure which can be predicted by the aid of Karl Pearson's tables. It naturally depends upon the size of the sample and the variation about the mean. Pearl defines it as a conventional measure of the reliability of re-

sults or of their "scatter" due to the chance effects of sampling. It is a constant so chosen that when its value is added to and subtracted from the average obtained for a particular sample the



chances are even that the true average lies on the one hand within the limits thus set by the probable error or, on the other hand, between these limits and infinity in either direction. Thus, we find from a study of the normal heart that the average number of fibers

per field is 11.5 ± 0.021. Then the mathematical probability that the true average for *all* the fibers of this heart of which we have studied a sample lies between 11.479 and 11.521, is exactly equal to the mathematical probability that the true average falls outside these limits. The significance of calculations upon this basis is furnished by the discussion of results of this study.

The breadth of the fibers
(in arbitrary units)

	Maximum	Minimum	Average
Normal heart	0.9	0.3	0.503 ± 0.0015
Atrophic heart	0.7	0.3	0.438 ± 0.0010
Hypertrophic heart	1.15	0.45	0.907 ± 0.0023

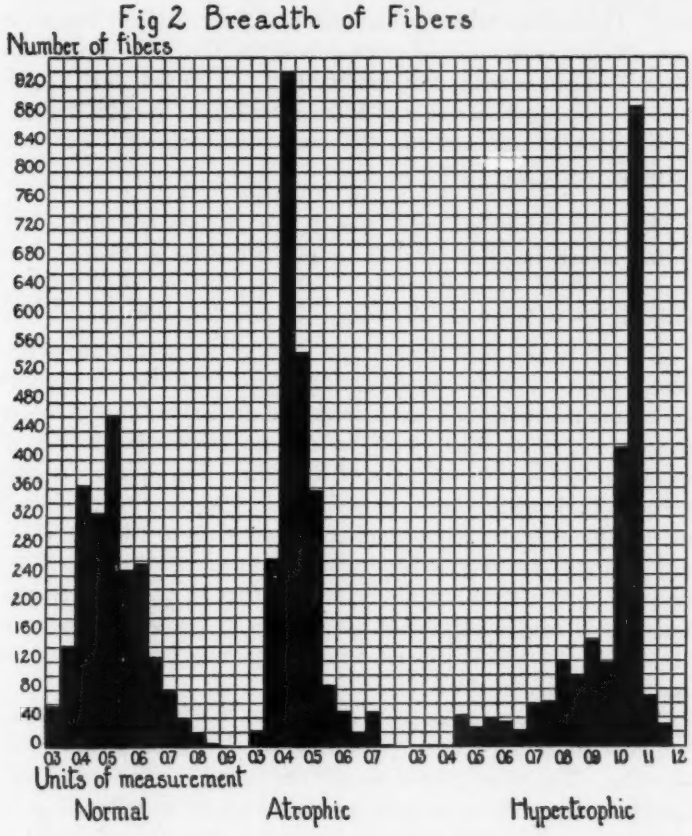
The number of measurements is so large, namely more than 2000 in each heart, that the error of random sampling is small and the results could not be significantly affected by further measurements, within reasonable limits. The measurements show differences in the

TABLE 2

The breadth of the fibers

Breadth of fibers (arbitrary units)	Number of fibers		
	Normal	Atrophic	Hypertrophic
0.3	54	22	0
0.35	142	264	0
0.4	372	940	0
0.45	328	546	46
0.5	467	348	28
0.55	251	86	38
0.6	258	52	36
0.65	128	20	26
0.7	82	52	60
0.75	45	0	62
0.8	20	0	124
0.85	5	0	102
0.9	1	0	156
0.95	0	0	110
1.0	0	0	418
1.05	0	0	890
1.10	0	0	72
1.15	0	0	38
	2,153	2,330	2,206

three hearts, with the same significance of results as exhibited by the number of fibers per field. An analysis of the figures is given in Table 2 and graphically represented in Fig. 2. In the normal heart the greatest number of fibers approximate in breadth the average. In the atrophic heart the greatest incidence is slightly to



the left of the average and in the hypertrophic heart distinctly to the right. As compared with the normal heart, the hypertrophic heart shows a much greater incidence of large fibers as well as many fibers larger than those in the normal. Conversely the atrophic heart shows a greater incidence of small fibers, but none smaller

than those observed in the normal (see Table 2). Since longitudinal splitting is said to occur in atrophic heart muscle it is possible that the increase in number of small fibers is due to this phenomenon, but if this were true there should be a considerable number of fibers smaller than those in the normal. No fibers of less than 0.3 unit (10.5 micra) were observed in either the normal or the atrophic heart. The number with this diameter is slightly smaller than in the normal heart, which suggests that in atrophy the fibers which were originally small undergo complete atrophy. By this method, as well as by calculating the number of fibers per field, the changes indicate an approach to uniformity in the phenomena of hypertrophy and atrophy.

Number of nuclei per field

	Maximum	Minimum	Average
Normal heart	26	5	11.0 ± 0.047
Atrophic heart	39	8	22.7 ± 0.075
Hypertrophic heart	20	5	9.8 ± 0.032

As would be expected the number of nuclei per field in the hypertrophic heart is less than in the normal heart. The number of nuclei per field in the atrophic heart is increased, but, as will be shown, in greater proportion than would be expected from the number of fibers per field and the breadth of the fibers. The figures are analyzed in Table 3 and shown graphically in Fig. 3. The number of fields containing the largest number of nuclei is to the left of the mean in the normal and hypertrophic hearts, and to the right in the atrophic heart. It is obvious, however, that in the hypertrophic heart fields containing a reduced number of nuclei predominate, whereas in the atrophic heart fields containing large numbers of nuclei are vastly in the majority.

The immediate problem is the question as to whether the increase in number of nuclei is due to crowding together or an actual multiplication. The most careful search has failed to disclose mitotic figures. A study of 29 atrophic hearts has shown no mitoses. It is, of course, possible that since the hearts are obtained post-mortem any mitotic figures present might have been completed. Subdivision of nuclei in various forms is present as shown in the photomicrographs, taken from the heart under special study. Similar pictures were found in 25 of the 29 other atrophic hearts examined. They may also occur in degenerations and in various forms of myo-

TABLE 3

Number of nuclei per field

Number of nuclei	Number of fields		
	Normal	Atrophic	Hypertrophic
5	3	0	18
6	30	0	110
7	116	0	288
8	370	6	466
9	292	6	392
10	350	0	416
11	183	6	290
12	247	8	220
13	158	46	200
14	108	58	70
15	100	106	74
16	43	104	14
17	33	150	8
18	25	124	8
19	26	158	4
20	25	182	6
21	10	102	0
22	8	146	0
23	5	136	0
24	3	152	0
25	4	174	0
26	4	132	0
27	0	110	0
28	0	98	0
29	0	94	0
30	0	136	0
31	0	10	0
32	0	72	0
33	0	18	0
34	0	22	0
35	0	44	0
36	0	10	0
37	0	6	0
38	0	8	0
39	0	9	0
	2,143	2,433	2,584

carditis. In lower forms amitotic division of cardiac muscle nuclei occurs (Conklin). We have observed mitotic figures in the fetal human heart but not in the adult nor in the dog heart. Herzog, how-

ever, claims to have observed both mitosis and budding in the heart muscle of victims of poisoning by illuminating gas. It is possible that these nuclear forms in the atrophic heart are of degenerative character, as observed by Van der Stricht and Todd in the conduction system, and the marked irregularity of the "corduroy" nuclei suggests that this is true. It might be thought that the vast increase in number of nuclei is due to counting the dumb-bell forms as two nuclei. Since the nuclei were counted with the 16 mm. objective an error of this kind is admittedly possible. Nevertheless, these forms constitute only about 2.5 per cent of the nuclei, which is of little significance in the 50 per cent increase observed.

For the purpose of demonstrating in simpler form the change in number of nuclei in relation to the fibers, a unit area may be assumed. The unit area is represented by the average number of fibers per field multiplied by the average breadth of fiber. The fiber unit area may be likened to a mat composed of fibers laid lengthwise side by side. Upon this mat the nuclei are scattered. The fiber unit areas for each heart are as follows:

Normal heart	5.75
Atrophic heart	4.75
Hypertrophic heart	6.64

By simple proportion, the number of nuclei in the hypertrophic and atrophic heart can be compared with the number in the normal heart. Assuming that there is no change in the distribution of nuclei upon the mat, the number of nuclei per fiber unit area should be 9.5 for the hypertrophic heart and 13.3 for the atrophic heart. The fiber unit area is for this purpose the effective field, since it disregards adventitious spaces. The observed average of nuclei per field in the hypertrophic heart is 9.8. This value is so close to the calculated value that it appears to demonstrate absence of change in relative number of nuclei when hypertrophy occurs. The observed number of nuclei per field in the atrophic heart is 22.7. Since the calculated value is only 13.3, it is apparent that the number of nuclei in the atrophic heart has risen to 150 per cent of the normal. As will be seen subsequently, the total number of fibers in the atrophic heart is reduced about 50 per cent. It might, therefore, be assumed that the increase in number of nuclei is apparent rather than real and due to a crowding together of the nuclei as atrophy progresses.

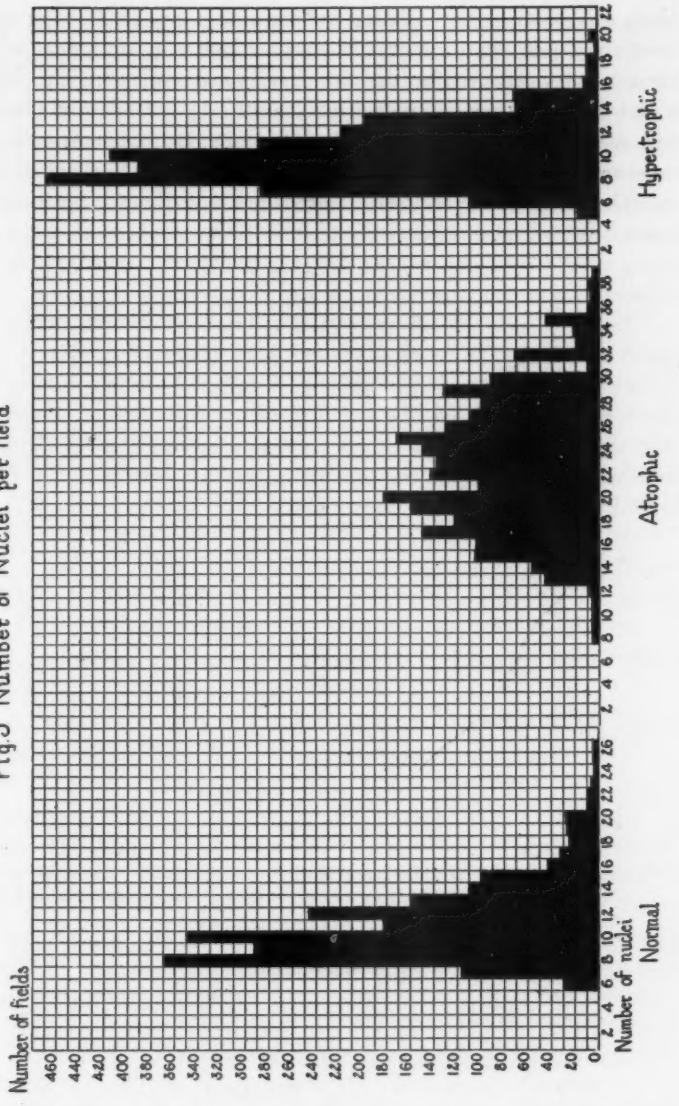


Fig. 3 Number of Nuclei per field

Since it is shown, however, that atrophy is accompanied by a loss of fibers this assumption would necessitate acceptance of the view that fibers may disappear and nuclei remain, which is not in accord with the generally accepted conception of cell form and nutrition. Furthermore, in the hearts studied all the nuclei counted were placed centrally in the fiber. The inference is plain that there must be in some way, either by mitosis or direct division, a multiplication of the muscle nuclei. This view has further support in the work of Edens, quoted above.

Discussion

The specific gravity of the normal and hypertrophic hearts is about the same, whilst that of the atrophic heart is slightly greater. Fibrosis is not a prominent feature in any of these hearts and if the alteration of specific gravity be significant, a condensation of solids must be assumed in the atrophic heart. With this difference of specific gravity and for other obvious reasons, it has seemed wise to attempt an estimation of total number of fibers upon the basis of volume rather than weight. The use of serial sections 5 micra thick makes it possible to calculate in terms of three dimensions. The figures arrived at are comparable but not to be regarded as absolute because of the syncytial character of heart muscle. The results are as follows:

Normal heart

Volume 295 c.c. Weight 300 gm.
Average 11.549 fibers in 0.00015 c.mm.
22,715,000,000 fibers in 295 c.c.
7,700,000,000 fibers in 100 c.c.

Atrophic heart

Volume 160 c.c. Weight 165 gm.
Average 10.801 fibers in 0.00015 c.mm.
11,520,000,000 fibers in 160 c.c.
7,200,000,000 fibers in 100 c.c.

Hypertrophic heart

Volume 485 c.c. Weight 500 gm.
Average 7.394 fibers in 0.00015 c.mm.
23,894,010,000 fibers in 485 c.c.
4,926,600,000 fibers in 100 c.c.

It is admitted that whatever error is present in the primary figures is multiplied in these estimates of the number of fibers in the hearts

or in the constant volume of 100 c.c. Nevertheless, it is probable that the total number of fibers in the hypertrophic heart is about the same as in the normal. In the atrophic heart, on the basis of this calculation and the others reported in the results of the study, the total number is reduced and this reduction is at the expense of the larger and smaller fibers. In the hypertrophic heart the individual fibers are large. Consequently, the number per 100 c.c. is reduced in proportion but the total number in the heart remains the same as in the normal. In the atrophic heart no fibers are found smaller than in the normal heart and the average fiber thickness is approximately that found in the normal heart. Hence, the number of fibers per 100 c.c. remains essentially the same as in the normal heart but the total number of fibers is reduced in general proportion to the smaller heart volume. It is suggested that there is a minimum to which reduction in size may go, beyond which the fiber rapidly disappears. The loss of transverse striation and vacuolization near the poles of the nuclei in the atrophic heart, as well as longitudinal splitting and reduced affinity for dyes, indicates a retrogressive change. This supports the studies of Bradley and his collaborators, who show definite autolysis in skeletal muscle. Perhaps the close relation of the number of fibers per constant volume to that of the normal heart is due to imbibition of water and the increased specific gravity due to a decrease in diffusibility of some of the products of disintegration.

Of further interest is the examination of variability. This is given in tabular form.

Variability of number of fibers per field

	Mean	Stand. Dev.	Coeff. of Var.
Normal heart	11.5 ± 0.021	1.45 ± 0.0146	12.61 ± 0.1294
Atrophic heart	10.8 ± 0.023	1.50 ± 0.0150	13.88 ± 0.1414
Hypertrophic heart	7.4 ± 0.015	1.07 ± 0.0104	14.46 ± 0.1441

This shows an essential uniformity of variability, from which it can be assumed that for all practical purposes the mean or average values really represent the number of fibers per field.

Variability of number of nuclei per field

	Mean	Stand. Dev.	Coeff. of Var.
Normal heart	11.0 ± 0.047	3.21 ± 0.0331	29.18 ± 0.3246
Atrophic heart	22.7 ± 0.075	5.65 ± 0.0546	24.89 ± 0.2551
Hypertrophic heart	9.8 ± 0.032	2.43 ± 0.0228	24.80 ± 0.2466

Variability of breadth of fibers
(in units)

	Mean	Stand. Dev.	Coeff. of Var.
Normal heart	0.503 ± 0.0015	0.102 ± 0.0010	20.404 ± 0.2186
Atrophic heart	0.438 ± 0.0010	0.072 ± 0.0071	16.409 ± 0.1690
Hypertrophic heart	0.907 ± 0.0023	0.160 ± 0.0016	17.550 ± 0.1826

In both these sets of figures it is found that the variability is greatest in the normal heart and definitely reduced in the hypertrophic and atrophic hearts. In other words both these changes tend toward greater uniformity. Variability is a characteristic of normality and constitutes Nature's provision for emergency. It seems to be fundamental to adaptability. In hypertrophy the response to extra demands for work appears to be in the production not only of large fibers but fibers with a greater degree of uniformity than exists in the normal heart. By reference to Table 2, this is seen to be due to an elimination of smaller fibers probably because they increase in size. In the particular heart studied 1528 fibers of the hypertrophic heart, or 69 per cent of the 2212 fibers measured, are larger than any observed in the normal heart. The number of nuclei per field is also more uniform.

In the atrophic heart the change is associated with retrogressive processes. This is represented by a reduction in the absolute number of larger fibers. In the normal heart, fibers with a diameter of 0.9 units (31.5 micra) are found, but the largest in the atrophic heart measure 0.7 units (24.5 micra). In the normal heart, 42 per cent of the fibers are less than the average of 0.5 units whereas in the atrophic heart 76 per cent are less than that figure. At the same time the coefficient of variability is reduced and this indicates a greater uniformity in size of fibers than in the normal heart.

The tendency toward uniformity as an adaptation to abnormal demands and conditions furnishes a new conception of the place of variability in adaptation.

The Study of Variation in Cardiac Muscle Fibers. In medical and biological studies the maximum and minimum values in addition to the average are stated in order to give some idea of the variability of the sample. The defect of this method is the apparent accidental nature of the precise maximum and minimum values noted. Further, these figures give no indication of the type of "scatter" about the mean value. In modern studies it is customary to replace the

maximum and minimum by a constant, known as the standard deviation. This is a quantity borrowed from theoretical mechanics in which it is known as the radius of gyration. For the purpose of this paper it is sufficient to state that within the limits of the standard deviation from the mean lie one half of all the individual units measured; in practice more than half may be found within these limits. None but "stragglers" lie beyond the limits defined by three times the standard deviation. Thus, in Table 1, the stragglers number six, eight and six in normal, hypertrophic and atrophic hearts respectively.

So long as it is desired to compare variability of a single character in several samples the standard deviation gives a convenient measure provided the mean values are approximately the same. Thus, the mean numbers of fibers per field in the normal and atrophic hearts are 11.5 and 10.8 respectively and we find these standard deviations are 1.45 and 1.50. But as soon as an attempt is made to compare variabilities in the same character when the means are widely divergent or when comparison is made of different characters the standard deviation fails to express clearly what the difference may be. Hence, a new value is calculated by ascertaining the percentage on the mean of its own standard deviation. This measure is the coefficient of variation. In the sample just quoted the standard deviations are respectively 1.45 and 1.50; the corresponding coefficients of variation are 12.61 and 13.88. When number of fibers per field is compared in the normal and hypertrophic hearts the means are quite divergent, namely 11.5 and 7.4. Their standard deviations, 1.45 and 1.07, do not readily disclose the precise difference in variation. Reference to the coefficients of variation defines this difference at once; it is represented by the figures 12.61 and 14.46.

Suppose one undertake, in the normal heart, to compare variability in number of fibers per field with number of nuclei per field. These are clearly different characters and the standard deviations, 1.45 and 3.21, are not directly comparable. But the coefficients of variation, calculated upon standard deviation and mean, at once make this comparison possible. Variability in number of fibers is 12.61 per cent and variability in number of nuclei is 29.18 per cent. It must not be assumed, however, that variability in nuclei is twice that of variability in fibers for, by the method of this investiga-

tion, fibers are counted in one dimension only of the field whereas nuclei are counted in two dimensions. In all such comparisons reservations must be made for the number of dimensions involved in the treatment of the character. This reservation can be mathematically computed (Miner). Comparing, in the normal heart, number of fibers per field with breadth of fiber one obtains the coefficients of variation 12.61 and 20.404 respectively. It is legitimate to assume that variability in fiber breadth is almost double that in number of fibers per field since both values involve merely a single dimension.

It is naturally desirable to have some absolute standard by which these variabilities may be judged. The simplest single-dimensional standard is the standing height of the body. The coefficient of variation for standing height in the male white dissecting room population has been ascertained to be 3.55 per cent (Todd). That a variability of 20.4 per cent should be obtained for breadth of normal cardiac fibers is by no means surprising, for the whole is less variable than its component parts. Indeed the restraint of variability in so minute a dimension is especially noteworthy.

Weight of the heart is closely related to volume and hence may be considered a three-dimensional character. The weight of the "normal" heart is given by Greenwood as having a coefficient of variation 17.71 per cent, that of the diseased heart has a variability of 32.39 per cent (Greenwood). The "normal" hearts are those which showed no evidence of disease at autopsy; the diseased heart series contained a large number of cases of valvular disorder as well as atrophic organs and this accounts for the relatively large variability. The three-dimensional variability of 17 per cent corresponds in practice to a single dimensional variability of about 8 per cent, and upon this standard the several variabilities recorded in this paper may be judged.

Comparison of Mean Values. To the mind unaccustomed to statistical treatment it may appear that the difference between some of mean values here recorded is so small that it should not be emphasized. But mathematically, the real value of the divergence may be gauged upon the theory of error. As an example, the difference between the mean values of number of fibers per field in the normal and atrophic hearts may be taken. The difference between the mean values is 0.7 (i. e., 11.5–10.8). The probable error of these averages depends upon the number in the sample and the variation in the

sample. Mathematically, it can be shown that the probable error of the difference between these averages is given by the square root of the sum of the squares of the probable errors of the two averages. One can, therefore, compute the relation of the difference between the averages to its probable error, or better to the standard deviation of the difference. By a simple technique this can be referred to the normal curve of probability, or, as it is often called, the Gauss-Laplace curve of error. Thus, one may calculate the chances that, in random sampling, the difference between the average values may or may not be reached or exceeded. In the example chosen the improbability of bridging this difference is represented by the figures $\frac{3.671}{10^{60}}$. This corresponds to odds so great that the certainty of a real difference in mean values is established beyond possibility of doubt.

Table 4 gives the result of an enquiry into the validity of the differences found between the mean values in all the previous tables. The odds in favor of a real difference between the mean values is so overwhelming that the validity of the difference cannot be disputed. Mathematically, it is found that a difference which is four times its probable error will arise less than once in one hundred trials. The odds in favor of a real difference between mean values, when this difference reaches eight times its probable error, are beyond any human power of conception. The results in Table 4 are given in terms of the standard deviation of the difference and this equals the probable error divided by the constant .6745. It is therefore about one and a half times the probable error. Hence, if the difference exceeds eight times its standard deviation the improbability of confusion in mean values is still more striking. To render more apparent the difference between mean values quoted in this paper there is added to Table 4 a statement of the ratio: difference between means divided by standard deviation. Comparison of these figures with the index of improbability indicates how rapidly the odds mount after the ratio difference divided by standard deviation reaches 3.

In this statistical survey it must be realized that mathematical presentation is merely a yardstick for measuring differences. Nothing can be obtained by mathematical treatment which was not there in the beginning. And some methods of treatment fail to bring out

the really significant facts. For example, Table 4 demonstrates that there is a real difference between the number of fibers per field in the normal and atrophic hearts, and again between the number of nuclei per field in the normal and hypertrophic hearts. It, of course, implies no more than this. Both these cases have been elucidated earlier in the paper. In the former case it has been shown that

TABLE 4

Improbability of confusion of mean values

	Improbability figure	Diff. / Stand. Dev.
Number of fibers per field		
Normal and atrophic hearts	$\frac{3.671}{10^{50}}$	15.2
Normal and hypertrophic hearts	$\frac{1.946}{10^{5322}}$	107.9
Number of nuclei per field		
Normal and atrophic hearts	$\frac{8.243}{10^{2051}}$	96.7
Normal and hypertrophic hearts	$\frac{7.794}{10^{45}}$	14.29
Breadth of fiber		
Normal and atrophic hearts	$\frac{2.781}{10^{127}}$	24.1
Normal and hypertrophic hearts	$\frac{1.344}{10^{2174}}$	100.0

the significant difference is the more uniform number of fibers per field (flatter curve) in the atrophic heart. In the latter case the genuine difference in number of nuclei can be discounted by reference to the fiber unit area.

SUMMARY

The enlargement of the heart in hypertrophy is due principally to a hypertrophy of the muscle fibers without an increase in the number of fibers. There is then no hyperplasia of the fibers and the process is one of pure hypertrophy. The change is accompanied by a distinct tendency toward uniformity in breadth of the fibers. The average breadth is significantly greater than in the normal heart. It would appear that all fibers tend to approximate the larger size and it is suggested that when all or nearly all the fibers have attained the maximum, further hypertrophy is impossible. What

determines the maximum is not disclosed by this study, but the anatomical basis for a "limit of hypertrophy" can be explained on this basis. Presumably, also, the relative reduction of reserve capacity in the hypertrophic heart may be due to the fact that the individual fibers have reached their maximum growth and probably also their maximum functional strength.

The reduction in size of the heart in atrophy is due to a reduction in size of the muscle elements, with a decrease in the number of fibers of the whole heart. The fibers are more uniform in breadth than in the normal heart. Since there are no fibers whose breadth is less than the narrowest fibers of the normal heart, the change must be due to an atrophic shrinkage and not to longitudinal splitting. The approach to uniformity is associated with a disappearance of the larger fibers, which have decreased in breadth toward the smaller average. Furthermore, many fibers have disappeared completely since the number of fibers in the entire heart is significantly decreased. All these changes are concordant with the conception of autolysis in atrophy as demonstrated by Bradley.

In proportion to the number of fibers the number of nuclei in the atrophic heart is vastly increased. This is clearly shown by mathematical calculations and is in accord with Edens's demonstration of relative increase of purine nitrogen in the atrophic heart. This might be regarded as an attempt at regeneration but no mitotic figures or clear indications of amitotic division were found. The increased number, however, is well established and must be due to some form of multiplication, but this study gives no information as to the fiber units (or cells) and does not correlate nuclei and fiber units. To elucidate the increased number of nuclei further work is necessary to demonstrate clearly and uniformly the intercalated discs.

The approach to uniformity in breadth of the fibers of the hypertrophic and atrophic heart as compared with the normal gives a new conception of variability as affecting the cardiac muscle in its adaptation to abnormal conditions.

REFERENCES

Aschoff, L. Pathologische Anatomie, Jena, 1921.

Bradley, H. C. Autolysis and Atrophy. Physiol. Reviews, 1922, ii, 415.

Chen, K. K., Meek, W., and Bradley, H. C. Studies on autolysis, xii, Experimental atrophy of muscle tissue. Jour. Biol. Chem., 1924, lxi, 807.

Collier, W. D. Adaptive changes of heart muscle. Jour. Med. Res., 1922, xliii, 207.

Conklin, E. G. Experimental studies on nuclear and cell division in the eggs of crepidula. Jour. Acad. Nat. Sci., Philadelphia, 1912, xv (ser. II), 503.

Durante, G. Du processus histologique de l'atrophie musculataire. Arch. Med. exp. et Anat. Path., 1902, xiv, 658.

Edens, E. Über Herzhypertrophie. Deutsches Arch. f. klin. Med., 1913, cxl, 288.

Foerster, A. Handbuch der speciellen pathologischen Anatomie. Leipzig, 1852.

Friedreich, N. Krankheiten des Herzens. Virchow's Handbuch der speciellen Pathologie und Therapie, Erlangen, 1861.

Goldenberg, B. Ueber Atrophie und Hypertrophie des Herzens. Virch. Arch. f. path. Anat., 1886, ciii, 88.

Greenwood, M. A first study of the weight, variability and correlation of the human viscera, with special reference to the healthy and diseased heart. Biometrika, 1904, iii, 63.

Herzog, G. Herzmuskeluntersuchungen bei Leuchtgasvergiftung Centrallbl. f. allg. Path., 1924, xxxv, 247.

Jamin, F. Experimentelle Untersuchungen zur Lehre von der Atrophie gelähmter Muskeln. Jena, 1904.

Kaufmann, E. Specielle pathologische Anatomie. Berlin and Leipzig, 1922.

Köllicker, A. Handbuch der Gewebelehre. Leipzig, 1852.

Miner, J. R. The variability of skull capacity. Jour. Phys. Anthropol., 1924, vii, 425.

Orth, J. Lehrbuch der speciellen pathologischen Anatomie, l, Berlin, 1887.

Paget, J. Lectures on Surgical Pathology. Philadelphia, 1865.

Pearl, R. Introduction to Medical Biometry and Statistics. Philadelphia, 1923.

Pearson, K. Tables for Statisticians and Biometricians. Cambridge, 1914.

Ricker, G., and Ellenbeck, J. Beiträge zur Kenntnisse der Veränderungen des Muskels nach Durchschneiden seines Nerven. Virch. Arch. f. path. Anat., 1899, clviii, 199.

Rindfleisch, E. Lehrbuch der pathologischen Gewebelehre. Leipzig, 1867.

Rokitansky, von C. Lehrbuch der pathologischen Anatomie. Vienna, 1856.

Schmidtman, M. Über feinere Strukturveränderungen des Muskels bei Inactivitätsatrophie. Centrallbl. f. allg. Path., 1916, xxvii, 337.

Tangl, E. Ueber Hypertrophie und physiologisches Wachstum des Herzens. Virch. Arch. f. path. Anat., 1889, cxvi, 432.

Todd, T. W. Thickness of the male white cranium. Anat. Rec., 1924, xxvii, 245.

Van der Stricht, O., and Todd, T. W. The structure of normal fibers of Purkinje in the adult human heart and their pathological alteration in syphilitic myocarditis. Johns Hopkins Hospital Reports, 1919, xix, fasc. 1.

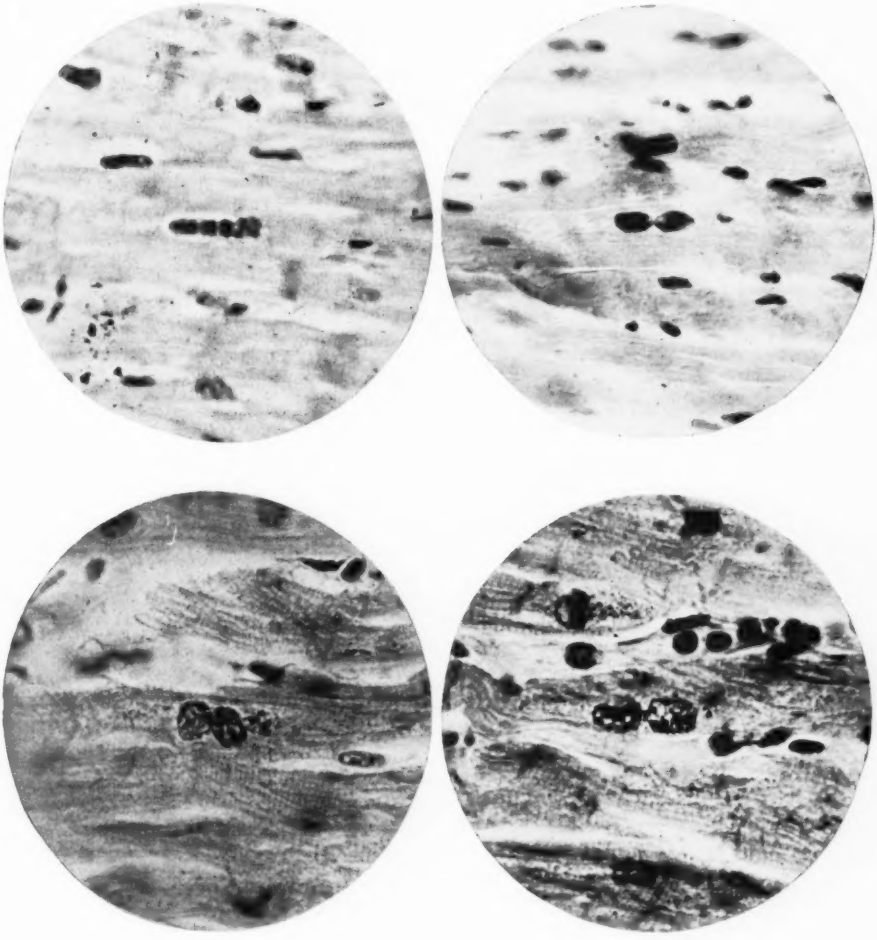
Vogl, J. Pathologische Anatomie. Leipzig, 1854.

Wideroe, S. Histologische Studien über die Muskulatur des Herzens. Virch. Arch. f. path. Anat., 1911, cciv, 190.

Zielonko, G. Pathologische Anatomie und experimentelle Studie über Hypertrophie des Herzens. Virch. Arch. f. path. Anat., 1875, lxii, 29.

DESCRIPTION OF PLATE LVIII

Photomicrographs (4 mm. obj.) of various forms of nuclei in the atrophic heart. Note the "dumb-bell" and "corduroy" forms.



Karsner, Saphir and Todd The cardiac muscle in hypertrophy and atrophy

THE HISTOLOGICAL ALTERATIONS OF THE PANCREAS IN CHRONIC PASSIVE CONGESTION *

WILLIAM C. VONGLAHN, M.D., and ROBERT CHOBOT, M.D.

(From the Department of Pathology, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City)

The purpose of this paper is to call attention to certain striking changes in the pancreas found in chronic venous congestion. The lesions associated with venous stasis in the lungs, liver, spleen and kidneys have separately been described; those in the pancreas are dismissed with the casual statement that they occur. We have found no detailed and minute description of these changes in the pancreas, either in the better known text-books or in the available periodical literature. To show how little attention has been paid to these lesions, the following references may be cited:

"Passive hyperaemia (of the pancreas) occurs in conditions of general passive congestion." (Adami and Nicholls.) [1]

"Longstanding difficulty of venous flow, due to chronic disease of the heart, liver, lungs, etc., may cause induration and fibrosis of the pancreas, as of other organs." (Mayo Robson and Cammidge.) [2]

"Among the secondary diseases of the pancreas must be mentioned metastatic tumors and abscesses; also hyperaemia and chronic interstitial inflammation, the result of venous congestion in chronic affections of the heart, lungs, or liver." "Passive hyperaemia may result from venous congestions, in consequence of heart, lung or liver disease; in such cases often combined with evidence of interstitial connective tissue, hyperplasia, and consequent increase of volume and consistence of the organ." (Friedreich.) [3]

"The frequency of chronic congestion of the abdominal viscera, and the relative infrequency of chronic interstitial inflammation of the pancreas, is evidence that the former condition is not commonly a cause of the latter. Chronic passive congestion may doubtless produce slight proliferation of the interstitial connective tissue, but is an unimportant factor in the production of chronic pancreatitis." (Opie.) [4]

* Received for publication April 12, 1925.

Our attention had for some time been attracted by atrophic changes in the pancreatic parenchyma occurring in cases of uncompensated cardiac disease with general venous stasis. In order to study this condition more systematically, and to establish, if possible, a precise relationship of these pancreatic changes to venous stasis, we have studied sections of the pancreas from 100 cases showing by histological examination chronic passive congestion of the liver, more or less intense. The pancreas in these cases was examined without reference to the clinical data. As control material 100 cases were selected in which there was neither clinical nor pathological evidence of long-standing impairment of the circulation. They were chosen from individuals of comparable ages.

In many instances only one section of the pancreas was available for study; in other cases there were sections from the head, middle and tail portions of the organ. The sections were examined with various stains: hematoxylin and eosin; Van Gieson's picro-fuchsin; Mallory's aniline blue-orange G and Heidenhain's iron hematoxylin.

Nature of the Lesions

Before analyzing this material in detail, the alterations which have seemed to us distinctive of chronic passive congestion of the pancreas may be described. The most striking feature, apparent at a glance, is an atrophy involving portions of each lobule (Fig. 1). While this atrophy does not affect the lobule uniformly, each lobule is to a greater or lesser degree involved; and in general the peripheral portion more than the central. It may be seen further that the islands of Langerhans are invariably surrounded by a zone of normal pancreatic tissue; and this is especially distinct in the most advanced cases in which only those acini in immediate contact with the islands show normal structure and dimensions (Fig. 2).

In contrast with the normal acini, in which the epithelial cells are ranged about a central lumen, and contain the normal prezymogen granules, the atrophic acini frequently show no lumen; the cells are reduced in size, their nuclei closely aggregated and shrunken (Fig. 3). The specific granules have disappeared.

There is no evidence that this atrophy leads to a true fibrosis of the organ as in some other tissues; the shrinkage of the parenchyma is followed or accompanied by a condensation of the connective tissue stroma and the basement membrane is not swollen. Very rarely

are a few small mononuclear wandering cells found in the atrophic areas. As Opie has already pointed out, there is no justification for the view of Friedreich and of Mayo Robson and Cammidge that chronic venous congestion may lead to fibrosis or chronic pancreatitis.

A point which must be discussed in this connection is the possibility that shrinkage during fixation may be confused with atrophy. It is impossible to avoid this artifact in routine post-mortem material no matter how careful the technique. There are, however, obvious differences between the artificial shrinkage and the tissue atrophy associated with venous stasis. In the former case, the cells are retracted from the basement membrane; in the latter, there is no retraction, the basement membrane shrinking with the acinus. Furthermore, the association of the atrophy with congestion of the capillaries and veins, and the more or less typical distribution of the atrophic areas, serve to eliminate the artificial effect produced during fixation. Finally, fixation shrinkage must have occurred with equal frequency in the control material, and yet as the tabulated observations show, none of these cases exhibited changes comparable to those found in chronic passive congestion.

Relation of the Lesion to Cardiac Decompensation

The cases comprising this series are readily divided into two groups: the first, those cases of long-continued or oft-repeated passive congestion associated with primary cardiac disease; and the second, cases of decompensation subsequent to diseases other than primary cardiac disease (Table 1).

In the first group are 72 cases and it will be noted that of these, 57 showed alterations in the pancreas.

Of the 28 cases in the second group, only 7 showed any changes in the pancreas which could be related to the passive congestion.

1. *Cases of primary cardiac disease with decompensation.* Further analysis of the histories of the 72 cases in this group was made to determine if there existed any relation between the time which elapsed after the first break in cardiac compensation and the degree of damage to the pancreas. Such study showed that there was no constant relation between the two, even though there may have been several periods of decompensation in the interval between the first break and the death of the individual.

More closely correlated with the severity of the lesion is the duration of the final period of cardiac failure. It was found indeed that

TABLE 1

		Atrophic changes in the pancreas			
		Slight	Moderate	Advanced	Negative
Cases of primary cardiac disease with decompensation	72	15	20	22	15
Cases of decompensation subsequent to diseases other than primary cardiac disease	28	4	2	1	21
Control cases	100	—	—	—	100

the extent of the damage to the pancreas is directly proportional to the length of the last break in the compensation of the heart. By reference to Table 2, it will be noted that no demonstrable change could be detected in the pancreas in those cases with an average

TABLE 2

Number of cases	Average duration of last period of decompensation	Changes in pancreas
15	10 days	0
15	33 days	slight
20	43 days	moderate
22	93 days	advanced

duration of 10 days; slight changes were present in those cases of 33 days; moderate, in 43 days, while in the cases in which the atrophy was advanced, the final period of decompensation was of a duration averaging 93 days.

In general it is found also that the alterations in the pancreas follow closely those existing in the liver. The more advanced the lesions of chronic passive congestion in the liver, the more intense were those in the pancreas (Table 3). There were, however, exceptions

to this finding, namely those cases in which the final period of decompensation was of short duration, and in these cases the altera-

TABLE 3

Chronic Passive Congestion of the Pancreas and Liver
Comparison of the Intensity of Alterations

Pancreas		Liver		
		Slight	Moderate	Advanced
Negative	15	4	6	5
Slight	15	1	4	10
Moderate	20	4	5	11
Advanced	22	0	7	15

tions in the liver were advanced in some instances while the pancreas showed no changes, or the alterations were slight (Table 2).

2. *Cases of decompensation subsequent to diseases other than primary cardiac disease.* Included in this group are a variety of diseases, such as chronic nephritis, etc.

The number of the cases showing alterations in the pancreas is too small to permit any conclusions to be made.

The question naturally arises whether it is possible to explain the distribution of the areas of congestion and atrophy in the pancreas on an anatomical basis. The study of the structure of the pancreatic lobule is necessary to answer this query.

In the human pancreas the distinct divisions as seen in the gross specimens are usually designated as lobules. These lobules are not individual units but are in reality made up of smaller masses of tissue which are described as the primary lobules. These primary lobules, not clearly separated from one another, are further united into groups varying much in size. Such lobule groups, or secondary lobules, are separated from each other by bands of loose connective tissue in which course the nerves, vessels and larger ducts. The secondary lobules are readily seen in the gross specimen and are grouped together to form the largest subdivisions or tertiary lobules. For the designation of these largest divisions some prefer the term

lobe of the pancreas. The islands of Langerhans in the human pancreas do not occupy any seemingly definite position in the primary lobules, though they are often situated near the centre.

The blood vessels accompany the ducts until the secondary lobule is reached. The arteries then no longer lie parallel to the ducts. In injected specimens a large artery can be seen penetrating to the central part of the secondary lobule where it divides rapidly and small branches ramify through the primary lobules. The veins run parallel to the arteries. Histologically it has seemed to us that the differentiation of arterioles from the smaller veins is one of great difficulty.

The circulation of the islands is of interest and importance with reference to the changes seen in chronic passive congestion. Many observers find a definite glomerular network in the islands. This network is made up of freely anastomosing channels much larger than capillaries and from these island vessels many capillaries pass between the acini immediately adjacent to the island. The nature of these vessels within the islands has been the cause of considerable discussion. von Ebner,[5] and De Witt[10] believe them to be venous; Kühne and Lea,[6] and Opie[7] think that they are closely connected with arterioles, though there is no definite afferent vessel to be made out. Opie,[7] in the pancreas of the cat, using granular injection masses through the arteries, found that the island vessels were filled and the interacinar capillaries contained little of the injected material; also, when a non-granular injection mass, such as Berlin blue, was used and the injection incomplete, that the islands often were injected and the nearby capillaries were for the most part empty. From these data he concluded that the glomerular network of the islands is in very free communication with the smallest arteries, and the blood supply to them is richer than it is in other parts of the lobule. Laguesse[8] states that generally a principal vessel enters the island, rarely an arteriole, generally a large branch continuous with an arteriole. Jordan and Ferguson[9] remark that "certain arterial branches also enter the islets and form a specially rich plexus of broad capillaries (sinusoids) within these cell groups."

De Witt,[10] working with the pancreas from many different animals as well as the human, came to the conclusion after double injections that the vessels in the islands were of venous origin, supporting the belief of von Ebner and others. Furthermore, in studying the pancreas from cases of chronic passive congestion, she found that there

was stasis of blood in the island capillaries and cites this in support of the venous origin of these vessels.

The areas of chronic passive congestion of the pancreas have been noted by us as being frequently at the periphery of the secondary lobule, the zone of congestion often extending toward the interior of the lobule. Small areas of congestion and atrophy occur also at various parts of the secondary lobule, being apparently quite irregular in distribution.

In reality, the position of these areas is not so irregular as would seem to be the case at first glance. When the changes are studied, beginning with those of slight degree, through the moderate to the advanced lesions, it becomes increasingly evident that these areas are found at the periphery of circulatory units. These circulatory units would appear to coincide with the primary lobules, the boundaries of which are sharply defined in the advanced cases by the ring of congested capillaries and atrophic parenchyma, with the island of Langerhans, surrounded by at least one row of acini with well-preserved cells, in the more central part (Fig. 2). This definition into lobules is best seen in the caudal portion of the organ, where the islands are most numerous.

The sinusoids of the islands of Langerhans are for the most part quite empty and we are unable to confirm the observation made by De Witt studying cases of chronic passive congestion. An occasional island is found with greatly engorged vessels or even hemorrhage but this ectasia occurs with equal frequency in the control cases and we do not consider it as distinctive of venous stasis. The engorgement of the capillaries becomes more marked as one passes outward from the islands, and this is especially well seen in the cases of advanced passive congestion (Fig. 2). Such findings would not be expected if the island vessels were of venous origin.

It is difficult also to escape the conclusion that the islands are closer to the arterial circulation and have a richer blood supply than the periphery of the primary lobule, for no changes have been detected in the island cells. Likewise those acini immediately adjacent to the islands escape injury because of their nearness to this better blood supply and their rich capillary anastomoses with the island vessels.

The distribution of the zone of congestion in the pancreas is quite comparable to that in the liver in severe chronic passive congestion,

the island of Langerhans occupying a position analogous to that of the portal area.

Summary

A comparative histological study of the pancreas has been made in 100 cases of cardiac decompensation and an equal number of cases in which there was no clinical or pathological evidence of chronic venous stasis.

In a large percentage of the cases of chronic passive congestion certain lesions are to be found in the pancreas, which are not present in the control series.

The distinctive features of chronic passive congestion of the pancreas are: (1) areas of capillary congestion at the periphery of the primary lobules, (2) atrophy of the parenchymal cells in the congested areas, (3) condensation of the connective tissue framework following or accompanying the atrophy of the cells, (4) disappearance of the prezymogen granules in the atrophied cells. It is also characteristic of this condition that there is no congestion of the vessels of the islands of Langerhans, no demonstrable change in the island cells, and no fibrosis in the congested portions.

The intensity of these changes in the pancreas follows closely that in the liver in chronic passive congestion, and is directly related to the duration of the final period of cardiac decompensation.

There is no evidence to support the belief that chronic interstitial pancreatitis follows chronic passive congestion of the pancreas.

We are indebted to Mr. Alfred Feinberg for the drawings.

REFERENCES

1. Adami and Nicholls. Principles of Pathology (second edition), 1911, ii, 498.
2. Mayo Robson and Cammidge. The Pancreas, its Surgery and Pathology, 1907, p. 165.
3. Friedreich. Diseases of the Pancreas. von Ziemssen's Cyclopaedia of the Practice of Medicine, 1878, viii, 586 and 590.
4. Opie. Diseases of the Pancreas (second edition), 1910, p. 235.
5. von Ebner. Archiv f. mikr. Anat., 1872, iii, 498. Kölliker's Handbuch der Gewebelehre des Menschen (sixth edition), 1902, iii, 254.
6. Kühne and Lea. Beobachtungen über die Absonderung des Pankreas. Untersuch. a. d. phys. Instit. d. Univ. Heidelberg, 1882, ii, 488.
7. Opie. Diseases of the Pancreas (second edition) , 1910, p. 66.

8. Laguesse: "Lobule et tissu conjunctif dans le pancréas de l'homme." Compt. rend. Soc. de Biol., 1905, lvii, 539.

9. Jordan and Ferguson. Text-book of Histology, 1916, p. 193.

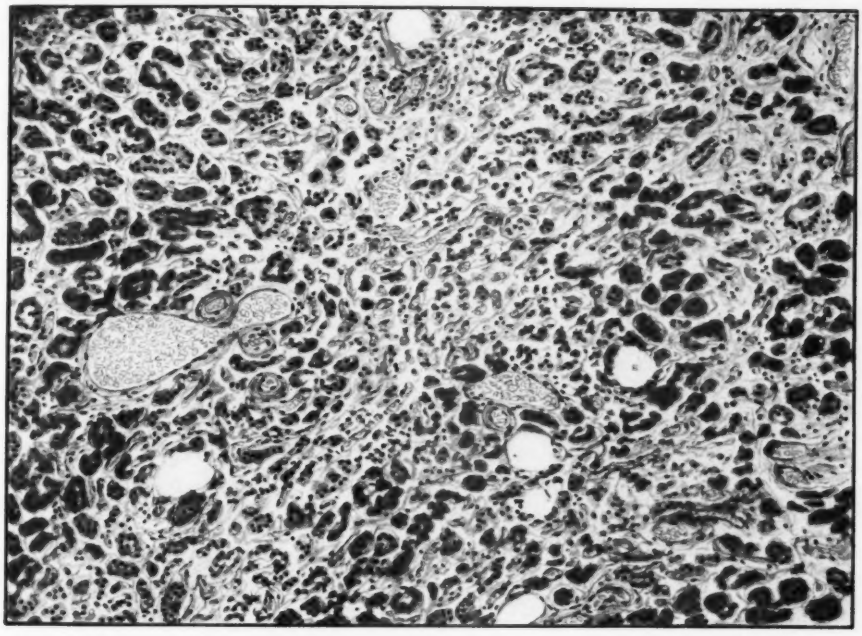
10. De Witt. Morphology and Physiology of Areas of Langerhans in some Vertebrates. Jour. Exp. Med., 1906, viii, 193.

DESCRIPTION OF PLATES LIX–LX

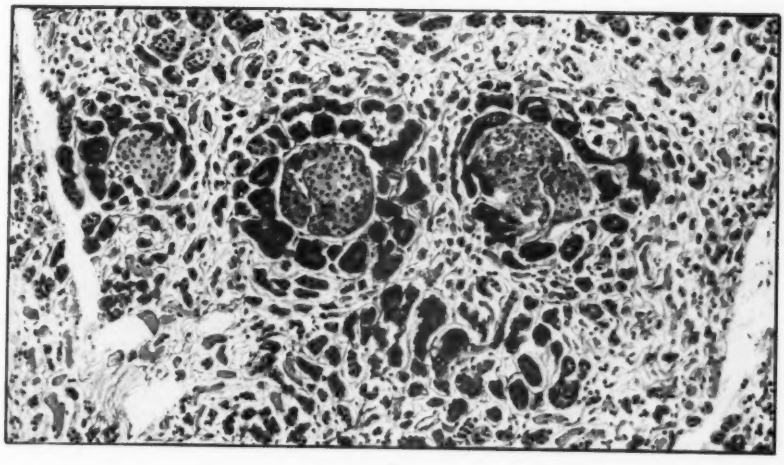
Fig. 1. Pancreas. Chronic passive congestion of moderate intensity. Atrophy of the parenchyma in the area of congestion.

Fig. 2. Pancreas. Extreme chronic passive congestion. Parenchymal cells well preserved about the islands of Langerhans. Marked atrophy of the cells in the peripheral portions of the lobules.

Fig. 3. Chronic passive congestion of the pancreas. A: Normal cells in an area where there is no congestion. B: Atrophy of cells in a congested area. (Photomicrographs from the same section.) x 450.

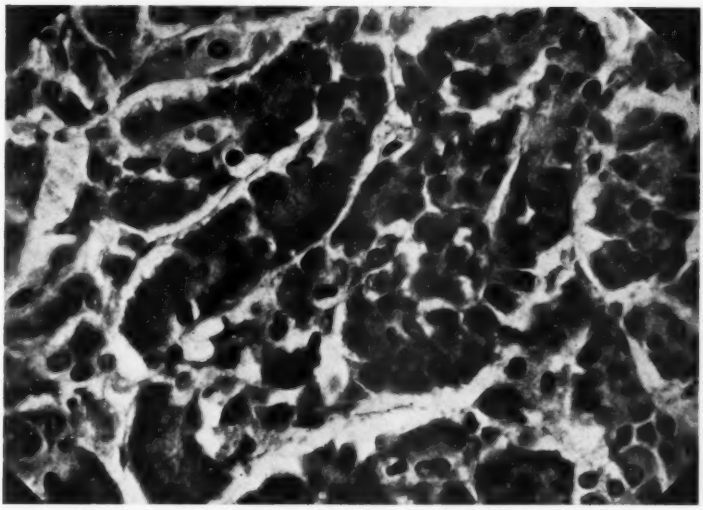


1

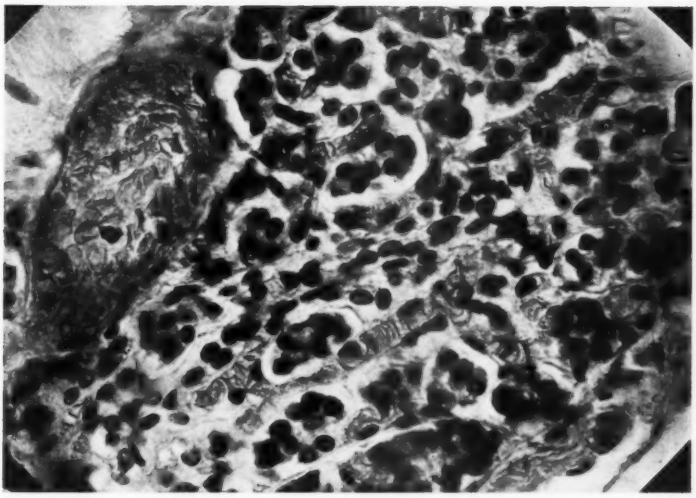


2

VonGlahn and Chobot Pancreas in chronic passive congestion



3



4

VonGlahn and Chobot Pancreas in chronic passive congestion

CHOLESTEROSIS OF THE GALL BLADDER *

STANLEY H. MENTZER, M.D.
(Fellow in Surgery, Mayo Foundation, Rochester, Minnesota)

The wall of the gall bladder is structurally well adapted for absorptive purposes. The mucosa of the gall bladder is formed of tall, thin, villous structures, intricate in their extensive branching, and delicate in the supporting framework of the stroma. If anatomic structure is the result of physiologic need, then certainly this mucosa is well formed for the end in view, that of intensive absorption. Indeed, physiologists have agreed that the gall bladder is essentially an organ of concentration. Rous and McMaster have proved definitely that water is absorbed from the bile in the gall bladder, thus concentrating the latter six to ten times. Moreover, physiologists have demonstrated the absorption of solid matter through the wall of the gall bladder. In 1920, Harer, Hargis and Van Meter injected potassium sulfocyanid into the cavity of the gall bladder of dogs and recovered the salt in the cystic node within five minutes after injection, thus showing the passage of particulate matter from the lumen, through the wall and thence into the lymphatic structures of the biliary system. In 1921, Boyd, after injecting potassium ferrocyanid into the lumen of the gall bladder of dogs, obtained the Prussian-blue reaction in the epithelial cells of the mucosa and even in the connective tissue cells of the stroma of the wall within thirty minutes.

I have repeated the work of these investigators and obtained similar results. Finely powdered carmine powder, charcoal and India ink were also used in separate experiments. Solid particles of these were found in the lymphatic structures within a few minutes after the injection into the cavity of the gall bladder.

The passage of solid substances, thus demonstrated, lends weight to the opinions of Grigaut, Boyd, Dewey, Starling and Sweet who believe that lipoids such as cholesterol are absorbed by the epithelial cells of the mucosa of the gall bladder. On the other hand, many

* Work done in the Division of Pathologic Anatomy, Mayo Clinic. Received for publication April 6, 1925.

workers, notably Adami, Herter and Workman, believe that cholesterol is excreted by the mucosa. A parallelism is drawn between epithelial cells of various parts of the body and those of the mucosa of the gall bladder, and the presence of cholesterol crystals in epithelial structures is cited. For instance, such crystals have been found in the bronchi and their presence attributed to "excretion" of cholesterol by the epithelial cells lining the bronchi. They have been found in leiomyomas of the uterus and attributed to similar excretion there. But it seems more reasonable to believe that the cholesterol crystals found under these circumstances are the result of cellular disintegration, as the cholesterol found in arteriosclerotic patches of the aorta undoubtedly is.

The experimental data recorded here are positive evidence of actual absorption of lipoid material by the epithelial cells of the mucosa of the gall bladder. So far as I know there is no other experimental evidence substantiating this work, but numerous investigators have said that cholesterol is probably absorbed by the wall of the gall bladder. In March, 1924, at a staff meeting at the Mayo Clinic, I reported the following experimental data:

Laparotomy was performed on twenty-three dogs anesthetized with ether. Into the cavity of the gall bladder of four of the dogs, finely powdered carmine suspended in distilled water was injected through a fine hypodermic needle in amounts of 10, 15, 20 and 25 c.c. These animals were necropsied within five minutes after the injections and in each carmine was observed in the cystic node. Other similar experiments were performed, powdered charcoal and later India ink being used in each case, with the same recovery of particulate matter in the cystic node. Therefore it seemed justifiable to conclude, as had previous investigators, that the wall of the gall bladder is capable of absorbing solid substances as well as fluids.

These findings led to experiments with other substances. The absorption of fats by the wall of the gall bladder proved an inviting problem and the experiments were repeated, using lipoid substances instead of particulate matter. Dogs were anesthetized with ether, and highly emulsified Sudan-stained fat particles injected into the cavity of the gall bladder. But in no instance was microscopically demonstrable fat discovered in the cystic node, even several hours after the injection. However, fat-stained particles in fresh untreated sections were present in the epithelial cells of the mucosa of

the gall bladder within a half hour after injection. And at varying intervals up to three hours, similar Sudan-stained globules were found in the connective-tissue cells of the stroma, and even in vascular endothelial cells. Similar observations were made by MacCarty in 1919 in a study of "strawberry" gall bladders. However, similar experiments with milk, olive oil and cholesterol esters were without success; in no instance was it possible to recover lipoid substances from the lymphatic structure of the wall of the gall bladder, even several hours after the injection.

However, the use of highly emulsified milk fat, deeply stained orange with Sudan III, produced astonishing results. Laparotomy was performed on anesthetized dogs and the fat-stained emulsion injected into the cavity of the gall bladder as in the previous experiments. Twenty-five minutes after the injection minute Sudan-stained globules were seen in the epithelial cells of the mucosa of the gall bladder, in otherwise unstained sections. And within one hour after injections these stained microscopic lipoids were seen as multiple, discrete masses throughout the epithelial cells, most numerous at the periphery of the cells, but present also at their base. In other sections, taken from the gall bladder of dogs necropsied two and three hours after injection, these tiny globules of previously stained fat were seen in the stroma and even endothelial lining of the blood vessels. Similar experiments, using cholesterol crystals dissolved in oleic acid, were not so satisfactory, but were sufficiently conclusive to verify the previous observations.

From these data, it was concluded that the wall of the gall bladder had the power to absorb lipoid substances, and that this absorption was effected, probably, by a direct passage of the lipoid through the epithelial cells of the gall bladder mucosa. Furthermore, it seemed likely that lipoid substances differed from particulate matter, in that they were not swept into the lymphatic stream, but were carried away through the blood vessels. At least, sufficient data were present to indicate that lipoid substances might pass through the wall of the gall bladder from its cavity.

The passage of lipoid through the wall of the gall bladder is probably a metabolic one, but our knowledge of the metabolism of lipoids is too indefinite to warrant any further speculation on this point. Suffice to say, physiologists are not in accord with the present theory of lacteal absorption of fats from the intestinal tract. Indeed, Mac-

Callum has proffered the suggestion that fats are probably absorbed directly by epithelial cells, and that this may be the method of their passage from the intestinal tract. However this may be, the factors concerned in such a passage are probably easily disturbed, and this disturbance would probably result in imperfect or incomplete passage of the lipoid through the wall of the gall bladder, with their resultant accumulation in the wall. Undoubtedly the lipoids would first accumulate in the epithelial cells of the mucosa, and later pile up in the connective-tissue cells of the stroma of the villi. Indeed, this is the characteristic picture of strawberry and papillomatous gall bladders on microscopic examination. The former shows the lipoid scattered diffusely over the surface of the mucosa, involving the distal or proximal portions of the epithelial cells and later the structure of the villi themselves. The papillomatous organ presents essentially the same picture, except that the areas of gross lipoid are localized in discrete polypoid masses.

However much we may be concerned with the direction of the passage of lipoid through the wall of the gall bladder, whether it passes from within the cavity into the wall, or vice versa, we cannot escape the fact that lipoids accumulate in the structures of the wall itself. Indeed, it is quite possible that the lipoid thus seen in the strawberry and papillomatous gall bladders is but an accumulation of cellular lipoid resulting from the degeneration of the cells themselves. It is difficult to believe such a contention, however, when one sees the relatively huge masses of lipoid that often distend the frail polypoid villi. It seems scarcely possible that such an amount of lipoid could come from the degeneration of the relatively few cells constituting their histologic structure.

Regardless of the method of accumulation, however, the fact is established that the gross appearance of the strawberry, fishscale and papillomatous gall bladders is due to lipoid substances piled up in the structures of the wall of the gall bladder, principally in the mucosa. MacCarty, in 1919, demonstrated that these lipoids are essentially cholesterin esters, and numerous observers have since verified this.

We are concerned, then, principally with an alteration in the cholesterol metabolism occurring in the wall of the gall bladder. The disturbance may be purely local, confined, so far as can be determined by our present methods, to the wall of the gall bladder itself.

That it is often associated with generalized disturbance in the fat metabolism of the body seems true.

In a study of 633 consecutive necropsies, in which I gathered the data myself, thereby reducing to a minimum the personal equation, 134 strawberry gall bladders, sixty-one papillomatous, and twenty-nine showing both conditions, were found. None of the persons was under fifteen years, and only three were under twenty; thus 34.7 per cent of adults showed this fat disturbance in the gall bladder. Also 21.6 per cent of the adults had gall stones, and 30 per cent of these also had fat alteration, making a total of 46.4 per cent of persons over the age of twenty that had some gross evidence of accumulated lipoid in the wall of the gall bladder. The lesion was found in 45.7 per cent of 150 consecutive men, whereas it was found in 53.9 per cent of women.

A majority of persons with fatty changes in the wall of the gall bladder also had fatty changes in other portions of the body. For instance, sixty-seven women had pathologic lipoid changes in the mucosa of the gall bladder. Fifty-eight of these (86.5 per cent) had been pregnant. It is well known that pregnancy is associated with fatty changes throughout the body, and that disease of the gall bladder frequently accompanies pregnancy. Likewise, the fat disturbance occurring in cases of obesity seems to be associated with the lipoid disturbance occurring in the wall of the gall bladder. In fifty-one necropsies, the subjects weighing over 200 pounds, 78.4 per cent had gross fatty changes in the wall of the gall bladder, and 94 per cent had abnormally large amounts of microscopic lipoid.

There is a slight increase in the frequency and amount of fatty changes in the liver in cases of lipoid disease of the gall bladder; the significance of this is not known. In 54.2 per cent of 315 cases in which there was no lipoid visible in the wall of the gall bladder, there were fatty changes in the liver, whereas in a series of 233 cases of strawberry and papillomatous gall bladders, fatty changes were present in the liver in 68.2 per cent. This is only a 14 per cent increase in the frequency of fatty changes in these cases, but the much greater extent and severity of the process was more striking.

That other organs in the body are concerned with these fatty changes is probable. It is known that certain organs contain relatively high proportions of lipoids, particularly the brain, adrenals, liver and blood vessels. Moreover, it is known that the cholesterin

content of these organs varies considerably, and probably fluctuates from day to day. Certainly it is true that the cholesterol content of the blood and bile varies from hour to hour and can be readily influenced by fat food intake. It seems likely that the various organs of the body are influenced by the lipoid content of the blood, and therefore change their lipoidal content as does the blood.

To facilitate the study of lipoid changes in the body, and especially that of cholesterol metabolism, and to obviate the rather unscientific terms of "strawberry" and "fishscale" gall bladders, a term descriptive of these changes has been sought. Inasmuch as it has been proved that the lipoid disturbance seen in the strawberry gall bladder, and the similar conditions, fishscale and papillomatous gall bladders, is essentially a cholesterol one, and inasmuch as the cholesterol content of the brain, adrenal, liver and blood vessels at least, undergo quantitative changes, it seems advisable to select a name, scientific and universally applicable, that will be descriptive of such alterations. These conditions are fulfilled by the term "cholesterosis," which is formed from the Greek "kolesteron" and "osis," meaning respectively "cholesterol" and "dysfunction." Our present knowledge does not permit further speculation on the type of changes that occur in the fatty derangements of the body. But we know that the pathologic changes here referred to are concerned with cholesterol, and the word "cholesterosis" simply indicates this dysfunction without further commitment. Regardless of what future studies may reveal as to the mechanism of this dysfunction, we know that the disease is concerned with cholesterol, and so the term will always be appropriate.

ARACHNID INFECTION IN MONKEYS*

(PNEUMONYSSUS FOXI OF WEIDMAN)

FERDINAND C. HELWIG, M.D.

(From the Department of Pathology, University of Kansas, School of Medicine)

During the course of routine autopsies upon monkeys used in the Department of Experimental Medicine at the University of Kansas, six animals from a consignment of nine were found to possess peculiar pulmonary lesions which were at first mistaken for tuberculosis. Further examination, however, showed them to be caused by an arachnid parasite which was discovered by macerating material from the lesions in a strong solution of potassium hydroxide and examining without staining. From every lesion examined one or more arachnid parasites were recovered.

Of the six animals possessing these lesions, two were used in the production of an experimental lobar pneumonia and one underwent an operation in which high obstruction of the ileum was performed. This animal died about 36 hours later. The remaining three showed no other gross lesions aside from the pulmonary infection. The monkey dying of intestinal obstruction showed in addition in the terminal ileum and colon, nodules varying in size from 6 to 14 mm. in diameter, extending above the surface on both the peritoneal and mucosal surfaces from 3 to 5 mm. From each of these nodules, nematodes were obtained which varied from 10 to 16 mm. in length.

Gross pathology. The lung lesions were subpleural, cone-shaped sacs which extended above the surface of the pleura from 0.5 to 1 mm. The larger lesions were slightly umbilicated on top, the smaller ones being smooth. They showed a yellowish gray center with a bright yellow border. The edges of the lesions were somewhat indurated and after opening revealed cavities with a thick wall of chronic granulation tissue partly filled with yellowish débris. Under the dissecting microscope, numerous arachnid parasites could be seen lying free in the cavity or attached to the walls, often

* Received for publication March 19, 1925.

surrounded by cheesy yellow material. The number of parasites in the cystic cavities varied considerably, as many as twenty being found in one of them. Each lung contained from twenty to forty lesions varying in size from 2 to 7 mm. in diameter on the pleural surface and extending into the substance of the lung from 3 to 7 mm. It was difficult to say whether they were in direct communication with the bronchi or not but careful dissection showed that in most cases the small terminal bronchi led up to the tip of the cone-shaped sac which pointed toward the hilum of the lung suggesting that the cyst was a primary dilatation of the bronchus in which the parasite had been lodged and which was shut off from the proliferating parasites by the inflammatory capsule formed.

Histological pathology. The microscopic study of these sacs shows that they have a more or less characteristic structure, being surrounded by atelectatic alveoli with thickened walls, blending into the substance of the cyst wall. The latter is composed of three layers, in most cases rather distinctly outlined but showing a tendency for the outer two layers to fuse together. The outermost layer is made up of some young connective tissue cells, fibroblasts and round cells in which there are scattered polymorphonuclear leucocytes. Rather large clumps of golden yellow pigment and dirty yellow pigment are irregularly distributed especially toward the inner wall. Many young capillaries, often filled with red blood cells are in the middle layer. The latter is much more cellular than the others, there being many large and small mononuclear cells and some polymorphonuclear leucocytes. The inner wall is again more fibrous in character there being a marked preponderance of fibroblasts and young connective tissue. Along the inner border parasites may be seen growing into the walls of the cavity. They are in many cases apparently becoming re-encapsulated by the connective tissue reaction. The intestinal canals of these parasites are filled with granules of yellow pigment indicating that they are probably parasitic excreta. This tendency to re-encapsulation is noted in numerous instances and is verified by the fact that in the larger cysts sectioned, the parasites are seen lying in small separate capsules in the immediate vicinity of the larger cysts. Although no organisms were found lying in bronchi which possessed intact bronchial epithelium the supposition that these organisms probably

do grow out from the bronchi is partially substantiated by the evidence of large amounts of pigment lying in the bronchi and by the encysted parasites growing out from the side of a bronchus. Both Weidman [12] and Landois and Hoepke,[11] who have described somewhat in detail cases of a similar character to these under discussion, did not find the parasites in the bronchi but Weidman [13] mentions a lesion, "abutting directly upon a bronchus with an especially necrotic wall."

The pulmonary lesions of the monkey showing the nematode infection of the gut present some scarring and more marked umbilication of the pleural covering of the sacs.

Section through these sacs shows an inner wall of more mature fibrous tissue than was noted in the previous cases and there is a more intense accumulation of round cells in the mid-zone with again a more adult fibrous tissue appearing in the outermost zone. There is a much larger accumulation of pigment in the vicinity of these sacs and it is here that the tendency to outpocketing is more noticeable than elsewhere. Some of the parasites are surrounded completely by a new capsule with a small communication which is not in any way large enough to permit entrance into the original or mother sac.

Mode of entrance. Our histological findings would tend to substantiate Weidman's assumption that the organisms probably gain access to the lungs through the air passages, lodge in the smaller terminal bronchi, proliferate, form sacs and from there burrow through into the interstitial tissue of the lung. The marked tendency on the part of the lesions to outpocketings, the parasitic excreta found in the bronchi and the suggestive location of the living organisms mentioned in the histological discussion seem to contradict the contention of Landois and Hoepke, that the ova are swallowed and gain entrance to the lung as embolic ova from the lymphatics of the intestine where they have burrowed and proliferated. As Weidman mentions the ova are of too large a size, measuring about 0.4 mm. in length, hence it seems probable that this latter source may be eliminated as a portal of entry. Further substantiation is found in Martin's case wherein he observed the mites crawling into the bronchi from the sac, and in serial sections he demonstrated bronchi leading into the subpleural cavities.

Classification. Although the organism somewhat resembles both that described by Hoepke and also Weidman, we felt that the matter of classification demanded the attention of an expert parasitologist and as a result some of the tissue was sent to Dr. H. B. Hungerford[17] of the Department of Entomology at the University of Kansas, who reported it to be the Pneumonyssus Foxi of Weidman. Before sending this material to Dr. Hungerford we thought that it might be of a somewhat different species than already described, due to the fact that the tissue reactions varied to some extent from those mentioned; this, however, might be accounted for by taking into consideration the age of the lesions. Dr. Hungerford sent some of this material to Dr. William A. Riley at the University of Minnesota who concurred with him in the diagnosis.

The following is a brief description of the organism after Weidman. "P. foxi n. sp.; diagnosis: Adult females, yellowish white, opaque, in width a little more than half the body length. Dorsal shield with ten hairs and pitted areas, ventral with six hairs. Anal plate present with three hairs. All tarsi furnished with two dorsal claws, all except leg i with caruncle in addition; all articles hirsute and most also spinulose. Both tarsi and femora subdivided. Palpi of three segments, all short, the terminal one capped by a short bristle. Mandibles chelate in both sexes. One pair of stigmal plates between and dorsal to coxae iii and iv. Hypostome bears a median longitudinal row of 9 to 13 teeth, carries ten hairs anterolaterally and four anterior marginal papillae. Vulva short, median and fissural at level of coxae iv. Adult males measure a little less than half as wide as long. Sexual orifice circular and close behind capitulum. Larva hexapod, oval, 0.55 × 0.28 mm., bears anal plate. Length 0.875 mm., breadth 0.478 mm.

"Habitat, lungs of monkey."

Discussion

Weidman does not feel that this organism is of high pathogenicity and fails to mention any pulmonary symptoms in conjunction with the infection. Three of our six cases, which did not show any other anatomical lesions at autopsy, during the course of their pulmonary infection were subject to very frequent attacks of paroxysmal coughing and sneezing, which we attributed to the arachnid infection. In addition, the fact that the lesions were more widespread

than those previously reported and that they caused, as we believed, such prominent respiratory symptoms, coupled with the additional finding of pulmonary edema in the uncomplicated cases, led us to conclude that they were of more than passive importance in bringing about the fatal termination.

Infection in man. It is of some interest to note that the arachnid infection of the internal organs of man is not, as is commonly believed, entirely unknown. A review of the literature brings to light a number of authentic cases and several in which the matter of contamination might be brought forward as an objection. The first case is that reported by Miyake and Scriba[1] in 1893, in which twenty-five mites and six eggs were found in the urine of a thirty-seven year old Japanese, suffering from hematuria. Marpmann[2] in 1898 found a solitary mite in the urine of a patient with gravel and albumin in his urine. Huber[3] in 1899 tells of three cases, one the case of Reinhart in which an arachnid was isolated from vomitus of a woman suffering with carcinoma of the stomach, the second that of Leroyde de Mericourt which showed parasites in the pus from a draining ear and the third that of Lambl's in which organisms were isolated from the stool of a patient suffering with dysentery. Troussart[4, 5] in 1900 discovered a male, female and larval arachnid in a testicular cyst which was of six years' duration. De Haan[6, 7] in 1906 found them in large numbers in the urine of one of his patients. Castellani[8] in 1907 while doing an autopsy upon a Beranda native in the Uganda discovered an arachnid parasite in a small cyst embedded in the omental fat. In 1910 Tieche[9] found them in the stools and urine in a case of erythema multiformis. Again in 1910 Tsunoda,[10] while examining a stool from an anemic patient in Japan, saw large numbers of arachnid parasites in all preparations studied. Recently (1921) Dickson[15] has reported two striking cases of mites in the urine, one in a patient with a severe cystitis and the other in which arachnids were said to be derived from the right kidney. The authenticity of some of these cases will perhaps be questioned but many of them seem to defy contradiction, especially those published more recently.

The possible advantage in reporting this apparent epidemic among a consignment of monkeys is to impress the possibility of internal infection by arachnid parasites in the human. The nature

of the lesion is essentially a local one suggesting the inability of the body to destroy the parasite and the necessity of surrounding it with fibrous granulation tissue to check its further spread. Undoubtedly the persistent cough of the animals was due to the irritation of these encysted organisms.

REFERENCES

1. Miyake, H. u. Scriba, J. Vorläufige Mitteilung über einen neuen menschlichen Parasiten. Berlin klin. Wchnschr., 1893, 374.
2. Marpmann, G. Über das Vorkommen von Milben im Harn. Centralbl. f. Bakt. Abt. I, Orig. Bd. 25, 1898, 304. Centralbl. f. Bakt. Bd. I, 1899, 25.
3. Huber, J. Ch., Bibliographie der klinischen Entomologie. H. 2. Acarinen, Jena, 1899.
4. Troussart, E. Faux parasit. d'une espèce de Sarcopt. détriticole dans un kyste du testicule chez l'homme. Compt. rend. Soc. Biol., 1900, lii, 742.
5. Troussart, E. Deuxième note sur l'Histiogaster spermaticus et sa prés. dans un kyste du testic. chez l'homme. Compt. rend. Soc. Biol., 1900, lii, 893.
6. Haan I. de, u. Grijns, G. Eine neue endoparasitäre Acaride. Centralbl. f. Bakt. Abt. I, Orig. Bd. 30, 1901, 7.
7. Haan I. de. Gibt es beim Menschen endoparasitär lebende Acariden? Centralbl. f. Bakt. Abt. I, Orig. Bd. 40, 1906, 693.
8. Castellani, A. Note on an acarid-like parasite found in the omentum of a negro. Centralbl. f. Bakt. Abt. I, Orig. Bd. 43, 1907, 372.
9. Tieche. Über massenhaftes Vorkommen von zur Familie der Tyroglyphiden gehörigen Milben im menschlichen Stuhl. Centralbl. f. Bakt. Abt. I, Orig. Bd. 54, 1910, 32.
10. Tsunoda, T. Eine Milbenhart von Glyciphagus als Endoparasit. Deutsche. med. Wchnschr., 1910, 1327.
11. Landois, F. u. Hoepke, H. Eine endoparasitäre Milbe in der Lunge von Macacus rhesus. Centralbl. f. Bakt. Abt. I, Orig. Bd. 73, 1914, 384. (Review of Literature.)
12. Weidman, F. D. Pneumonyssus Foxi, nov. sp., An Arachnoid Parasite in the Lung of a Monkey (Macacus rhesus). Jour. Parasit., 1915, ii, 37.
13. Weidman, F. D. An Arachnoid (Pneumotuber macaci, Landois and Hoepke?) Parasitic in the Lungs of a Monkey (Macacus rhesus). Jour. Comp. Path. & Therap., 1915, xxviii, 326.
14. Duncan, F. M. On Acari from the Lungs of Macacus rhesus. Jour. Royal Micr. Soc., 1920, 163.
15. Dickson, W. E. C. Mites as Internal Parasites of Man. Jour. Trop. Med., 1921, xxiv, 25.
16. Fox, H. Disease in Captive Wild Animals and Birds, 1923. Philadelphia, Lippincott Co., pp. 647–648.
17. Hungerford, H. B. Personal communication.

DESCRIPTION OF PLATES LXI–LXII

Fig. 1. Photograph of pleural surface of lung, light area at apex shows external appearance of lesion. Natural size.

Fig. 2. Photograph of cross section through lung showing two communicating subpleural sacs. Natural size.

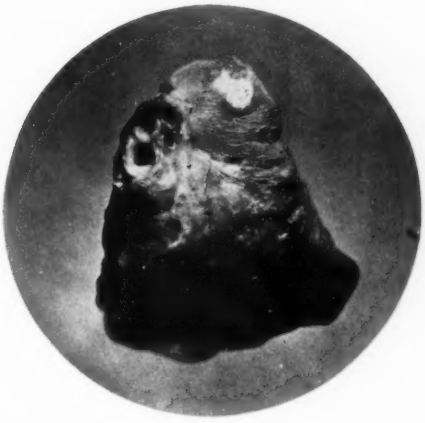
Fig. 3. Photomicrograph of section showing organism burrowing into wall. H and E stain. x 80.

Fig. 4. Photomicrograph of immature mites. x 60.

Fig. 5. Photomicrograph of section through a sac showing organisms lying within. x 8.

Fig. 6. Photomicrograph of six-legged Larva. x 60.

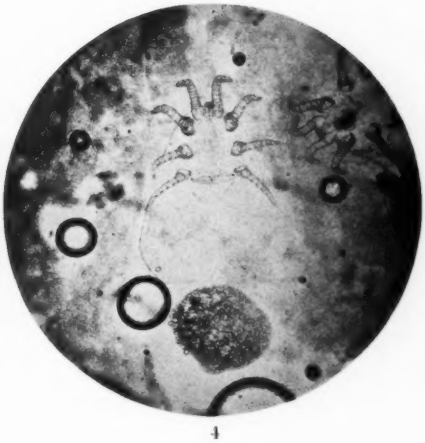
Fig. 7. Photomicrograph of adult female Arachnid. x 100.



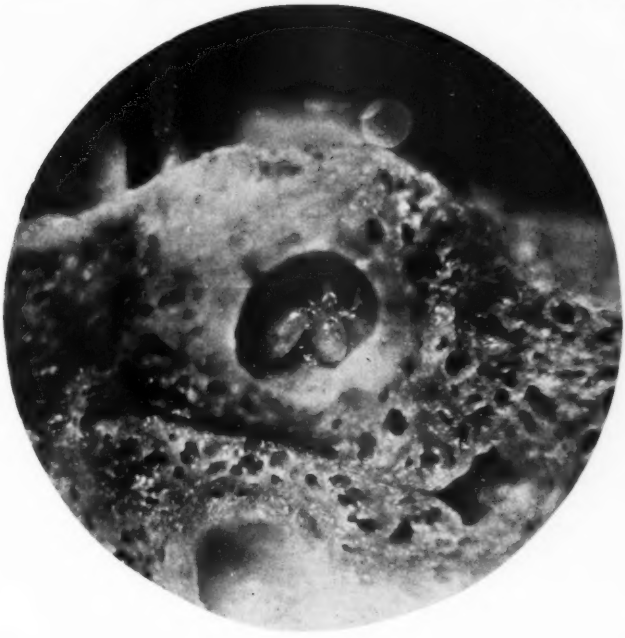
1

2

3



4



5

6

Helwig

7

Arachnid infection in monkeys

MUCOCELE OF THE APPENDIX WITH GLOBOID BODY FORMATION *

GIBBS MILLIKEN, M.D. AND C. A. POINDEXTER, A.B.

(From the Laboratory of Pathology, Medical Department, University of Texas, Galveston)

Cystic dilation of the vermiform appendix is an infrequent but interesting pathological entity, of which 168 cases are recorded in the literature. A more rare form of the same condition is the so-called "fish-egg" or "globoid body" type, of which there are only six cases reported. Because of its rare occurrence we wish to record another interesting example illustrating this unusual disease.

Latham [1] reported a case which consisted of a dilated appendix 9 cm. in length. There was no opening between the appendix and cecum, the normal orifice having been closed by an obliterative enteritis. The structure was found to be filled with small round transparent bodies the size of dried peas. Sections revealed a fibrous-like structure with an irregular distribution and laminated in parts, being more laminated at the circumference where it had the appearance of a capsule. These sections stained with acid dyes and gave no reaction for mucin; they did not stain by the Weigert method for fibrin. The wall of the appendix was irregularly honey-combed by the pressure of the bodies; it was stiff and thickened, the mucous membrane had almost disappeared and no lymphoid tissue remained. The condition had given no symptoms during the life of the patient who was a man aged 46. He died of lobar pneumonia.

Another example of this same lesion is mentioned by Cobbett and Keilin.[2] Dr. John Pooley of Nottingham sent the specimen to them and appended a brief statement of the case. The patient was a soldier who had died of carcinoma of the intestine with metastases in the pancreas and suprarenal bodies. There were no signs or symptoms of a diseased appendix and it was opened at necropsy only because it was distended. "It was so kinked that its cavity was quite shut off from the rest of the bowel." Upon opening the appendix the content was found to be many small grayish-white bodies

* Received for publication April 11, 1925.

that were at first considered to be parasites. They resembled frogs' eggs very closely, except that the nuclei were whitish rather than pigmented, and they were not adherent one to another. They appeared spherical when floated in a weak solution of formalin, but some of them were drawn out to a blunt stalk-like point on one side. The size varied, the majority measuring from 2.5 mm. to 3 mm.; some were even 4 mm. in diameter. Their outline was sharply defined; in the middle of each could be seen an opaque white nucleus, measuring about half the total diameter, and outside this, a peripheral zone which was gray and translucent. Sections of these bodies showed that they were devoid of structure, beyond the fact that the nucleus was denser than the remainder and torn away from the peripheral zone.

A second case was noted by Keilin [3] in the First Eastern Hospital, Cambridge, during the war. This was found at autopsy in a soldier who had died of an infected wound. The appendix had given no signs or symptoms during life. The writers do not give a detailed description of this case.

Shattock [4] reported a case in a male, age 23 years, who had symptoms of appendicitis and was operated upon. The appendix was removed. It was somewhat enlarged and was tightly packed with semi-transparent spherical bodies "like the roe of a fish." There were numerous patches of inflammation on the peritoneum. When crushed and stained with iodin the bodies exhibited groups of oval nuclei distributed in a homogeneous matrix. Serial sections including the wall of the appendix and the adjacent contents led this writer to state, "There is a catarrhal inflammation, accompanied by a hyper-secretion of mucus and desquamation of the proliferating epithelial cells, the mucin affecting the form of spherules as though extruded from and accompanied by a destruction of the crypts."

Morrison [7] recorded another very interesting example of this type of mucocele, discovered while operating upon a patient for chronic cholecystitis. The appendix was found to be markedly dilated, extending into the true pelvis.

The gross description of the appendix given by him is as follows: "The appendix appeared whiter in color than the normal; it was of firm consistence, as if distended with a solid substance. There was a uniform enlargement from base to tip, and its diameter was as large as the width of the small intestine. A few adhesions were pres-

ent about the appendix. At its base, where it joined the cecum, there was a definite constriction of the appendicular walls. The mesentery appeared normal."

The pathological report of the specimen made by Dr. F. B. Mallory of the Boston City Hospital is as follows: "The length and width of the gross specimen measured 5.5 centimeters long by 2 centimeters in diameter at its base, and 1.5 centimeters in diameter at its tip. A transverse incision was made at the middle of the appendix, and another longitudinal incision was carried through its walls opposite the mesentery. The lumen was distended with a quivering, jelly-like mass, embedded in which were innumerable small rounded nodules, varying from a translucent to a white color and up to one millimeter in diameter, the whole resembling fish roe; the substance was not attached to the wall of the appendix, merely distending its lumen. When dropped into alcohol, formaldehyde-alcohol, Zenker's and Klotz's fluids, the little bodies immediately shot away from the mass. The appendiceal wall was about one to two millimeters thick, pale and white; it had two firmer nodular thickenings, each about four millimeters in diameter, at the mesenteric attachment.

"The microscopic examination of the wall of the appendix in its thinnest portion showed almost complete atrophy of the mucosa, with disappearance of the epithelial elements. No inflammatory reaction was evident. The muscle coat was intact, but stretched and thinned. The small balls of secretion were composed of masses of necrotic cells embedded in mucus. A section through the proximal end of the appendix showed the mucosa present, but the glands were obliterated, probably owing to the stretching which had taken place. The lining epithelium was thickened to form a stratified layer from three to eight cells in thickness. The cells were of cylindrical type, and a few of them were multinucleated (half a dozen to a dozen nuclei in each). The process resembled that sometimes seen in the gall bladder when stones are present.

"The specimen represents an advanced stage of cystic dilatation of the appendix, in which not only mucus is found, but also little balls of mucoid material, which may later become calcified, as well as the walls of the appendix itself. For descriptive purposes it may be called a fish-egg mucocele."

We are also indebted to Dr. F. B. Mallory for directing our atten-

tion to the reference to another case of this variety of mucocele that was reported by Cagnetto,[8] a very similar type of lesion to that found by Morrison in his case. This was also an accidental finding and the patient had no symptoms referable to the vermiform appendix.

To the foregoing cases we wish to add one for which we are indebted to Dr. R. W. Noble of Temple, Texas.

Case Report

Mrs. A. G. Age 32 years.

Summary. Recurrent right lower abdominal distress; tenderness upon deep pressure over McBurney's point; left lower abdominal pain; surgical intervention with removal of a simple cystic left ovary, chronically inflamed right fallopian tube and dilated appendix filled with "globoid bodies" composed of pseudomucin; lumen of appendix closed at cecal junction.

The patient was admitted to the King's Daughters Hospital, Temple, Texas, on March 7, 1923. She complained of an attack of pain in the right iliac region. This pain was a recurrent one felt at intervals for several years. There was pain and tenderness in the left side of the lower abdomen.

The physical examination revealed some pain upon deep pressure over McBurney's point. A bimanual examination of the pelvic structures brought out the presence of an enlarged and painful cystic ovary on the left, as well as a laceration of the cervix and a laceration of the perineum.

The white blood count was 5,400. There were 64% polymorphonuclear leucocytes. There was not an eosinophilia.

A cystic left ovary, the right fallopian tube that was the seat of chronic inflammation and a very markedly dilated vermiform appendix were removed at operation by Dr. R. W. Noble.

The patient made an uneventful recovery, and when seen by Dr. Noble in July, 1923, she was in perfect health.

Pathological Report

The appendix is larger than normal, being 10 cm. long and 4 cm. in diameter at the widest point. It is tapered from the cecal end toward the tip. (Fig. 1.) The peritoneal surface is smooth and has retained the normal sheen. The structure is entirely filled with small globoid bodies that are semi-transparent. Many of these bodies have a denser, whiter central spot; further, all of them have a tapered tip giving the appearance of small pears. The size of these bodies varies widely, the smallest being no larger than the head of a pin, while the largest is 1 cm. in diameter and 0.9 cm. in length. No connection exists between the various structures as is evidenced by

their freedom when placed in water. Chemically we find that they are composed of pseudomucin.

The appendix and the globoid bodies were fixed in formalin. Sections stained with hematoxylin and eosin bring out the fact that the wall of the appendix is greatly thickened, the chief constituent being fibrous tissue, which in some places has become hyalinized. Very little remains of the mucosa and the submucous coat is very much thickened and infiltrated with small mononuclear leucocytes. The serosa is not remarkable. (Fig. 2.)

The globoid bodies have been studied by serial sections and their structure found to be of granular and fibrillar form. (Fig. 3.) The granular material takes the basic stain suggesting a chromatin nature while the fibrillar portion has an affinity for the acid dye. An attempt to section one of the bodies and the appendiceal wall at the same time proved to be a failure, the friability of the tissues being too great.

The central opaque area seen in the gross examination of these bodies is not of different nature from the peripheral zone, but is more compact.

Discussion

The etiology of mucocele of the appendix is rather obscure. Elbe [5] has given some underlying conditions that are essential for its development.

First, a slowly stenosing process at one or more points of the lumen. A rapid stenosis produces gangrene.

Second, a sterile lumen must be present distal to or between the points of stenosis. Bacterial invasion, if of sufficient virulence, would cause a secondary empyema of the appendix.

Third, there must be an actively secreting mucosa, or at least a more rapid secretion than absorption, and also some change in the mucosa whereby the normal mucus is transformed into pseudomucin.

In the globoid body form these features are essential but an additional one is also present, the mucoid material being excreted from the crypts, as suggested by Shattock.[6] An inflammatory reaction is of considerable importance as a stimulus to hypersecretion of mucin, and all of the cases collected that were studied microscopically presented this feature except the one reported by Morrison.

Conclusions

1. Mucocele of the appendix is very infrequent.

2. The globoid body type of mucocele of the appendix makes up only 0.35 per cent of the total number of reported cases.

3. This unusual form most probably represents a combination of secretion of mucus into the crypts and some disturbance of reabsorption of the secreted material, plus a chemical change whereby normal mucus is transformed into pseudomucin.

4. Less than half of the reported cases had symptoms referable to the appendix during life, the condition being frequently an accidental finding at necropsy or during an exploratory abdominal operation.

REFERENCES

1. Latham, A. Trans. Path. Soc., London, xlviii, 86.
2. Cobbett, L., and Keilin, D. Mucoid Casts from the Vermiform Appendix. Journal of Path. and Bacteriology, xxvi, 297–299.
3. Keilin, D. Loc. cit.
4. Shattock, S. G. Proc. Roy. Soc. Med., 1920, xiii; Path. Sect., Pt. iii, 106.
5. Elbe, quoted from Davidson, Marshall. Mucocele of the Vermiform Appendix. International Clinics, 32d ser., ii, 1922, 303–309.
6. Shattock, S. G. Loc. cit.
7. Morrison, W. R. "Fish-egg Mucocele of the Appendix; A Cystic Type of Chronic Catarrhal Appendicitis, with Report of a Case." Boston Med. and Surg. Jour., 1923, clxxxviii, 532.
8. Cagnetto, G. "Über einen eigentümlichen Befund bei Appendizitis." Virchow's Arch., Berlin, 1909, cxcviii, 193.

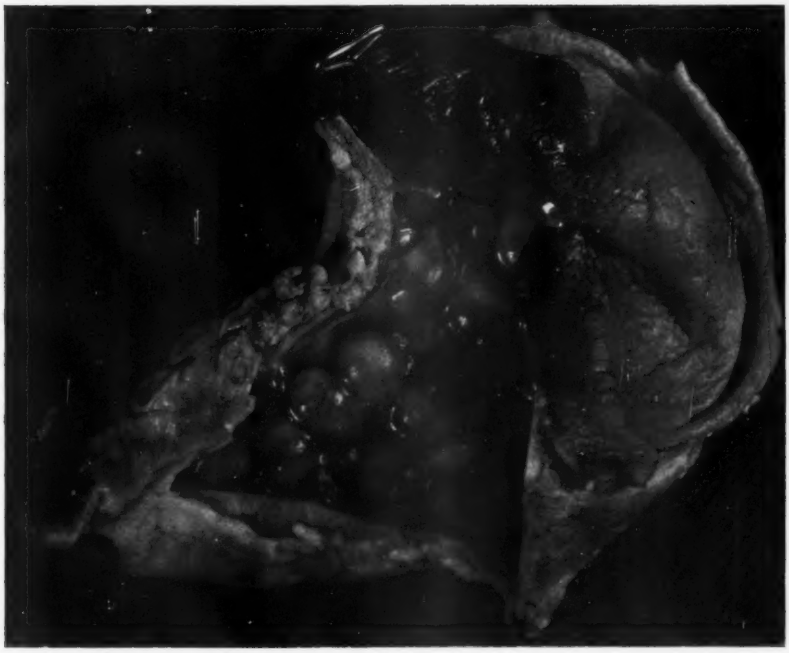
DESCRIPTION OF PLATES LXIII–LXIV

Fig. 1. Globoid bodies in a mucocele of vermiform appendix. (Case of Milliken and Poindexter.)

Fig. 2. Section through the wall of the appendix showing a crypt filled with mucoid material. (High power photomicrograph.)

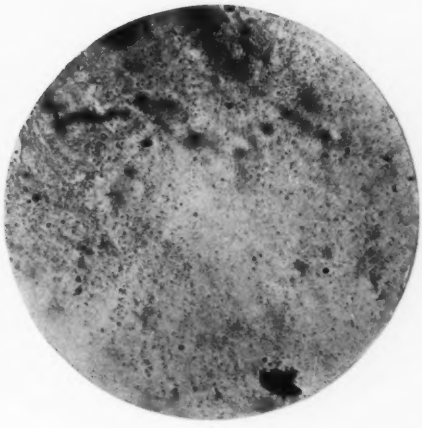
Fig. 3. Section of a globoid body demonstrating the granular structure of this. (H. & E. stain, high power photomicrograph.)

Fig. 4. Fish-egg or globoid body mucocele in the vermiform appendix. (Case of Morrison.)



1

2



3

Milliken and Poindexter

Mucocele of the appendix

4

Milliken and Poindexter Mucocele of the appendix

THE EFFECT OF CERTAIN METABOLIC CHANGES UPON THE AORTA OF RABBITS AND GUINEA PIGS *

OTTO SAPHIR, M.D.

(From the Department of Pathology, School of Medicine, Western Reserve University, Cleveland, Ohio)

Introduction. There are recorded in the literature a great number of attempts, with varying success, to produce experimental arteriosclerosis in animals. Numerous investigations have been conducted on an empirical basis. Many of the experiments which have been successful in the production of arteriosclerosis have at least one factor in common: that is, the arterial lesions have been observed in animals following the ingestion or injection of substances which alter metabolism. There would therefore appear to be some possible relationship between metabolic alterations and the experimental production of arteriosclerosis.

Pick and Pineles [1] reported that the production of arteriosclerosis in animals by adrenalin is favored by the feeding of thyroid substance. Murata and Kataoka [2] observed that the adrenalin type of arteriosclerosis occurred frequently in animals fed on thyroid substance. It has been demonstrated by Williams, Riche and Lusk [3] that protein feeding definitely increases the metabolic rate and Newburgh and Clarkson [4] have reported that rabbits fed on a high protein diet show a tendency for the development of arteriosclerotic lesions. Anitschkow [5] has shown that the administration of cholesterol, which is a metabolic stimulant, may produce arteriosclerosis in animals without the occurrence of cholesterolemia.

The following study was undertaken to observe on the aortae of animals the effect of repeated administration of substances which alter metabolism, over a prolonged period of time.

Experimental. Three substances were employed, each of which has somewhat different effects. Thyroxin was used for its stimulating effect on metabolism. Sodium acid phosphate was used to produce acid intoxication, at first in doses which Spiro [6] found sufficient to produce acid intoxication and subsequently in larger doses.

* Received for publication April 12, 1925.

Quinine hydrochloride was used in doses equivalent to 20 grains for man, in other words large doses which alter metabolism probably with depression.

Thyroxin tablets (Squibb) were given by mouth in doses of 0.2 mg. per kilo of body weight and the crystals intravenously in doses of 0.1 mg. per kilo of body weight. Sodium acid phosphate was injected intravenously in doses of 1 c.c. of a 3 per cent solution per kilo body weight, although later this was increased to 5 c.c. of a 3 per cent solution. Quinine hydrochloride was injected intravenously in doses of 20 mg. per kilo of body weight dissolved in a 3 per cent solution of urethane as advised by Sollmann.[7]

Observations were made on rabbits and guinea pigs. The animals were weighed before and at the end of the experiment. All animals that died during the course of the experiment were excluded. Since arteriosclerotic lesions occur frequently in rabbits, 34.77 per cent according to A. B. Miles,[8] 50 rabbits and 30 guinea pigs were used as controls. The aortae of all animals were fixed in 10 per cent formalin, embedded in paraffin and stained with hematoxylin and eosin. Blocks were taken from the ascending and descending portions of the thoracic and from the abdominal aorta.

Results

Thyroxin. Ten rabbits and 8 guinea pigs were each fed 0.2 mg. per kilo of body weight by mouth daily for 60 days. Sixteen rabbits were each injected intravenously with 0.1 mg. per kilo of body weight daily for 60 days. Half of the animals of each group were killed following the last dose while the remaining half were killed two months later. The aortae of all animals were examined and neither gross nor microscopic evidence of arteriosclerosis was found. All animals showed a loss of weight. Those which were injected showed in most instances a loss of about 30 per cent although one small animal lost only 8 per cent. Those fed with thyroxin lost from 11 to 16 per cent with one exception which lost only 2 per cent. The loss of weight of the guinea pigs ranged between 16 and 48 per cent.

Sodium Acid Phosphate. Twenty rabbits were injected intravenously daily over a period of 90 days with the following doses:

Oct. 1 to Nov. 9	1 c.c. of 3 per cent aqueous solution
Nov. 9 to Dec. 9	3 c.c. of 3 per cent aqueous solution
Dec. 9 to Dec. 19	4 c.c. of 3 per cent aqueous solution
Dec. 19 to Dec. 29	5 c.c. of 3 per cent aqueous solution

Ten of the rabbits were killed following the last injection and the remaining 10 were killed 2 months later. There were no gross or microscopical arteriosclerotic changes. The loss of weight was in the neighborhood of 10 per cent, the smallest loss being 6 per cent. Guinea pigs were not used in this series because of the difficulty of intravenous administration over a long period.

Quinine Hydrochloride. Twenty-one rabbits were injected intravenously and 8 guinea pigs subcutaneously with 20 mg. per kilo of body weight each day for 30 days. At that time the necrosis at the site of injection was so severe as to interrupt the experiment. Seven of the rabbits and 4 of the guinea pigs were killed following the last injection. Fourteen of the rabbits and 4 of the guinea pigs were killed two months later. In the abdominal aorta of three animals were found small, elevated, yellow, smooth, plaques. Upon microscopical examination hyperplasia of the intima was seen with fatty changes of the hyperplastic intima and sub-intimal layers of the media. The rabbits each lost about 20 per cent in weight and the guinea pigs about 20 per cent.

Controls. Fifty rabbits and 30 guinea pigs were examined. These were apparently normal, non-experimental animals. The rabbits ranged in weight from 1200 grams to 3900 grams and the guinea pigs from 250 grams to 680 grams. In none of the guinea pigs was there any evidence of arteriosclerosis. In the descending thoracic aorta of one and the abdominal aorta of two of the rabbits there were found arteriosclerotic lesions similar to those seen in the animals that received quinine. Microscopically, there was in one case calcification of the media of the aorta. In all three aortae there was intimal hyperplasia with fatty changes of the hyperplastic intima and sub-intimal layers of the media.

Summary

There were no arteriosclerotic changes in the aortae of the animals receiving thyroxin or sodium acid phosphate. In 3 of the rabbits injected with quinine and in 3 of the control rabbits, early arteriosclerosis of the aorta was observed. The laboratory conditions are such that none of the control animals lost weight during the period of the experiment. All the experimental animals lost weight but this can be due to loss of appetite, frequent handling, etc., quite as well as to altered metabolism.

It is safe to assume that the arteriosclerosis in three experimental animals was not due to the treatment. Therefore, in the stock employed in this laboratory about 6 per cent exhibit non-experimental arteriosclerosis.

Since thyroxin failed to produce arteriosclerosis, it seems probable that cholesterol arteriosclerosis is not due *per se* to increased metabolism.

Conclusion

Under the conditions of the experiments here recorded, the metabolic disturbances incident to the prolonged administration of thyroxin, sodium acid phosphate and quinine hydrochloride do not produce arteriosclerosis of the aorta of rabbits or guinea pigs.

Grateful acknowledgment is made of the aid rendered by Professor T. Sollmann and Dr. Alan R. Moritz.

REFERENCES

1. Pick, E. P., and Pineles, F. Ueber die Beziehungen der Schilddrüse zum Gefässsystem. Verhandlungen des Kongresses für innere Medizin, 1908, xxv, 360.
2. Murata, M., und Kataoka, S. Experimentelle Atherosklerose und Schilddrüsenfütterung. Verhandlungen der japanischen pathologischen Gesellschaft, 1918, viii, 221.
3. Williams, H. B., Riche, J. A., and Lusk, G. Metabolism of the dog following the ingestion of meat in large quantities. Jour. Biol. Chem., 1912, xii, 349.
4. Newburgh, L. H., and Clarkson, S. The production of arteriosclerosis in rabbits by feeding diets rich in meat. Arch. Int. Med., 1923, xxxi, 653.
5. Anitschkow, N. Zur Ätiologie der Atherosklerose, Virch. Arch. f. path. Anat., 1924, ccxli, 73.
6. Spiro, K. Beiträge zur Lehre der Säurevergiftung beim Hund and Kaninchen. Beiträge zur physiolog. Chemie, 1901–02, i, 269.
7. Sollmann, T. A Textbook of Pharmacology. Philadelphia and London, 1922.
8. Miles, A. B. Spontaneous arterial degeneration in rabbits. Jour. Am. Med. Assn., 1907, xlix, 1173.

STUDIES ON PNEUMONIA FOLLOWING NASO-PHARYNGEAL INJECTIONS OF OIL *

G. F. LAUGHLEN, M.D.

In the routine study of autopsy material at the Toronto, Ontario, Hospital for Sick Children, an unusual microscopic picture was observed in sections of the lung from a pneumonia case. Two days later a similar observation was made in a second case.

Brief abstracts from the histories of these cases are as follows:

Case I. This child, aged 2½ years, had an illness lasting about seven weeks. She first developed diphtheria, from which she recovered after four weeks illness, and about one week or ten days later she developed scarlet fever which was complicated by Vincent's angina. She eventually developed septicaemia and died.

She was only four hours in the Hospital for Sick Children, during which time she received sod-diarsenal intravenously, 15 minims of camphor in oil hypodermically and a transfusion of blood. Menthol in alboline † had been used as treatment of her nose and throat infection previous to her admission to the hospital. Argyrol drops had also been used in the same way but a record of the amount given could not be obtained.

At autopsy grayish red nodules of broncho-pneumonia were found in all lobes of the lung excepting the left upper lobe. There was nothing unusual in the distribution or gross appearance of the broncho-pneumonic process.

In sections of the consolidated portions of the lung, the alveoli were seen to contain three types of exudate: one in which polymorphonuclear leucocytes predominated, another consisting almost exclusively of large vacuolated mononuclear cells, and a third type in which both of these cells were intermixed.

Attention is particularly called to the mononuclear cells which made up this peculiar type of exudate and which contained unstained droplets of various sizes. Some cells contained so many droplets that they were distorted in shape and considerably swollen. A few contained no droplets but the majority contained amounts intermediate between these two extremes.

By the use of Sudan III these globules were demonstrated to be oil. These "oil containing" cells did not appear to be undergoing degeneration but were healthy, as shown by the good nuclear stain-

*Read before the Section of Pathology, Academy of Medicine, Toronto, Ont., Nov. 25, 1924. Received for publication April 3, 1925.

† Menthol — grs. I. — Alboline oz. I.

ing even of those cells which were loaded with droplets. In those which contained much oil the nuclei were often pushed to one side, flattened, or somewhat masked. In others which contained very little the nuclei were round or oval and centrally located.

The appearance and phagocytic nature of these cells characterize them as endothelial leucocytes.

Case II. This infant had an illness lasting seven weeks which commenced with a cough and naso-pharyngitis, and which was followed in one week's time by double suppurative otitis media. In the course of her illness conjunctivitis and convulsions also developed. There was a profuse nasal discharge.

Local treatment consisted of instillations of argyrol drops in the eyes and three drops of menthol and alboline in each nostril every four hours. The menthol and alboline treatment was used for thirty-six days and until three days preceding her death.

On post-mortem examination there was well marked broncho-pneumonia of the right upper lobe and both lower lobes of the lung. As in the preceding case no unusual features were seen on gross examination of the lung, but on microscopic examination, in addition to an exudate of the usual septic type, extensive areas were found in which the pneumonic exudate consisted almost exclusively of large phagocytic endothelial cells (Fig. 1). These were loaded with droplets which were demonstrated to be oil by the use of Scharlach R. stain.

Case III. This child, three years of age, was a diabetic and in the course of her treatment she received two drachms of paraffin oil as a laxative. She was in a somewhat stuporous condition when this was administered and two days later she died in coma.

Post-mortem examination of the lungs showed broncho-pneumonia of both the lower lobes.

In the sections of the lungs oil containing endothelial cells were observed in great numbers in many of the alveoli. An ordinary septic pneumonia was also present.

On reviewing the autopsies of the previous year, about three hundred in all, another case was found in which oil had been administered through the nose and which showed the typical oil phagocytes in the lungs.

A condition similar to that in the four preceding cases was found in an adult at the Toronto General Hospital.

A man aged thirty-seven years had an illness of two years' duration which consisted of multiple paralyses of the face, arms, legs, soft palate, vocal cords and accessory muscles of the larynx. He also had dysphagia, but could swallow if food were placed well back in his mouth.

He received 1.5 oz. of liquid paraffin three times a day over a period of four and one-half months. It was administered by mouth as a laxative.

Twelve days after this treatment was discontinued he had an elevation of temperature and two weeks later showed cyanosis and physical signs of broncho-pneumonia. Eleven days later he died.

At autopsy there were areas of consolidation in all the lobes of both lungs, and the cut surfaces exuded an oily liquid.

Microscopically in addition to a septic pneumonia great numbers of the alveoli were filled with endothelial cells containing oil and in places large droplets of free oil were also observed. This stained fairly well with Scharlach R. stain.

It has been customary in many children's hospitals and among pediatricians in private practice to treat nose and throat infections by the use of drops of menthol and alboline or by the use of argyrol drops. In this paper only the five cases which gave histories of "oil treatment" are referred to in the discussion which follows.

From a study of these cases it was apparent that the endothelial cell reactions in the lungs were due to oil but it still remained to explain the source of the oil and the route by which it reached the lung alveoli and also to prove that oil would produce such reaction in the lungs of animals.

The first experiments with animals were undertaken to prove if possible that the administration of oil led to reactions in the lungs of animals similar to those seen in the clinical cases.

Menthol and alboline was administered in several ways in order to study the route by which it found its way into the lungs.

It was also necessary for comparative purposes to repeat the same experiments using a 15 per cent watery solution of argyrol instead of the menthol and alboline.

For Experiments 1, 2, and 3, three healthy rabbits were selected.

Experiment 1. One-half c.c. of menthol and alboline was injected by means of a syringe and small catheter which was passed into the trachea a distance of about 5 cm. through a tracheotomy wound in the neck. Two days later 0.5 c.c. of menthol and alboline was again injected in the same way and twelve hours after this injection the animal died.

A post-mortem examination showed the cause of death to be broncho-pneumonia. Streptococcus hemolyticus and staphylococcus aureus were recovered from the lungs. The lung alveoli and bronchioles contained an exudate of red blood corpuscles, polymorphonuclear leucocytes and mononuclear cells. In some of the alveoli, vacuolated endothelial leucocytes were present. These were shown to contain oil by the use of Scharlach R. stain. Though few in number they were of the same type as those observed in the clinical cases.

Experiment 2. One-half c.c. of menthol and alboline was administered intratracheally by means of a sterile syringe and needle every second day for one month. Altogether fifteen injections were given. The animal was then killed and examined.

The lower lobes of the lung appeared deeply congested. They were air-containing and crepitant throughout and floated readily in water. On microscopic examination, many of the alveoli, especially throughout the lower lobes, were seen to contain large phagocytic endothelial cells. The droplets within these cells gave the reaction for fat. There were no polymorphonuclear leucocytes present in the exudate. The cells within the alveoli were of the same type and character as those seen in the clinical cases (Fig. 2). The lesion appeared to be produced purely by the presence of oil and was not obscured by reaction to secondary infection.

The rabbit of Experiment 3 received 1 c.c. of menthol and alboline every second day for one month and 1 c.c. daily thereafter until it died. This was injected into the pharynx by the use of a syringe and small catheter. The catheter was inserted into the mouth a distance of about two to three inches to insure the oil being retained. No attempt was made to insert the catheter into the larynx and the opening of the catheter was at the side which rendered it unlikely that any oil would be suddenly thrown into the larynx; moreover the animal was not manipulated in any way and the oil was given with the animal on its back but it was allowed to turn over after each injection.

This rabbit died (on the day I had planned to kill it) two months and six days after the experiment was commenced. The post-mortem examination showed that death was due to broncho-pneumonia. Both lower lobes of the lung were consolidated and mottled in appearance. The greater part of these lobes was dark red in color, but here and there somewhat lighter grayish-red areas were seen. At the margins of the lower lobes and in the upper lobes, bright red areas could be seen which contrasted sharply with the darker red color of other areas. This bright color did not fade on fixing in formalin but became much more distinct, as the color of the surrounding lung tissue faded. This color was due to Scharlach R. which had been added to the last eight or ten doses of the menthol and alboline which were administered to the rabbit. The quantity of Scharlach R. was just sufficient to give a bright red color to

the oil. This served to identify on gross examination the presence of the stained oil, widely distributed throughout the lung.

These features can be made out in the gross specimen. The red color is still bright although the lungs have been kept in formalin since April 21, 1924.

On microscopic examination, a widespread broncho-pneumonia was seen throughout all lobes of the lung. The reaction was of a mixed type but the bulk of the exudate consisted of endothelial cells containing oil droplets (Fig. 3). In many places no other cells were present but areas could be found in which the exudate consisted largely of polymorphonuclear leucocytes.

Two other experiments were carried out before the termination of Experiment 3. It was the purpose in these experiments to set up a throat infection which would reproduce more closely the conditions present in the clinical cases before oil was administered.

Two healthy adult rabbits were selected and their throats swabbed with 50 per cent carbolic acid. The following day both rabbits showed a severe local reaction and were breathing with difficulty.

One rabbit was then given 0.5 c.c. of menthol in alboline dropped into the pharynx and the same amount again on the following day. This rabbit died on the third day and on examination showed a well-marked broncho-pneumonia of the septic type and numbers of oil-containing endothelial cells within some of the alveoli.

The other rabbit was kept as a control and received no treatment. This rabbit recovered.

No definite conclusions have been drawn from these two experiments because the rabbit that died had a slightly more severe reaction from the phenol than had the control rabbit. The experiments are included to show that the oil had found its way into the lung alveoli although only two 0.5 c.c. doses were given.

Experiments were undertaken using argyrol 15 per cent instead of menthol and alboline. This was considered necessary because argyrol as well as menthol and alboline had been administered in one of the clinical cases which has been described, and because argyrol has been extensively used in the treatment of nose and throat infections.

Two healthy adult rabbits were used in these experiments:

One rabbit received daily intratracheal injections of 0.5 c.c. of argyrol which was introduced through a sterile syringe and needle.

This rabbit died from broncho-pneumonia on the third day but no noticeable endothelial cell reaction was found in the lungs.

The other rabbit received 0.5 c.c. of argyrol daily, introduced into the pharynx by a syringe and catheter in the same way as the menthol and alboline of Experiment 3. This rabbit died in two months and twenty-five days. It was much emaciated and the kidneys showed a very advanced nephrosis. There were also degenerative changes in the liver, but no endothelial cells were found within the lung alveoli.

Discussion

The phagocytic cells which have been especially noted in this paper have been referred to as endothelial cells. They are apparently the only ones concerned in the disposal of oil droplets. From a study of the literature it seems clear that they are identical with cells phagocytic for coal dust and other solid particles: the so-called "dust cells." They are also the same as the endothelial cells of pneumonic exudates, the epithelioid cells of tubercles and the heart-failure cells of passive congestion of the lung.

It is also clear that besides possessing marked phagocytic power, they all possess considerable power of ameboid movement. Their origin has long been in dispute. In the present state of our knowledge there is no reason to believe that they arise from the lining cells of the alveolar walls.

Permar [1] has shown that they arise largely from blood vessels of the alveolar walls and perhaps from vessels of more distant parts such as the liver and spleen. He found that under the stimulus of foreign bodies in the alveoli the endothelial cells of vessels proliferated, developed power of ameboid movement, entered the alveoli and became actively phagocytic.

The reaction in the lungs of animals following oil administration has been shown to be identical with that seen in children.

In three of the children the only way the oil had been given was by nasal drops which were allowed to run back into the nasopharynx while the children were in bed and on their backs.

The quantity of oil in two of these cases was considerable: 6 drops every four hours or about 2 c.c. a day for a considerable period of time. In another case only a single dose of 2 drachms was given by mouth.

In the five clinical cases and two of the animal experiments, oil found its way into the terminal air passages though it was only injected into the nose, mouth or pharynx.

In the experiments where oil was administered by mouth as well as in the clinical cases the reactions to oil were complicated by septic pneumonia, but in Experiment 2 in which oil was injected by needle into the trachea a purely endothelial cells reaction with no complicating sepsis was set up.

In one rabbit a granulomatous tumor developed at the side of the mouth where the surface was saturated with oil from the injections.

These observations suggest that oil, by lowering the resistance of the tissue or by carrying infection from the nose and throat, may be a factor in the production of pneumonia.

The oil used for mouth injection and intratracheal injection was the same. It was not sterilized so that accidental infection from outside may have occurred; but in that case the rabbit of Experiment 2, that received oil by intratracheal injection and in which more oil must have entered the lungs, should have developed a septic pneumonia more readily than the others. This points to the infection being autogenous in spite of the contention that the organisms ordinarily present in the throats of man and animals are incapable of producing infection in the host.

In Experiment 3, in which unstained oil was administered for nearly two months and oil stained with Scharlach R. was given throughout the last week or ten days, there was considerable unstained oil within the lungs, showing that oil had been reaching the lungs in the earlier part of the experiment. This oil could have carried infection equally as well as the oil given in the last ten days. Apparently the animal did not develop septic pneumonia until a large number of its lung alveoli were filled with endothelial cells and oil.

The reactions in these cases were produced by mineral oils but similar reactions have been observed in the lungs of animals following intratracheal injections of olive oil.[2,3]

The movements and final disposal of the oil phagocytes are subjects for further study.

This paper has called attention to a type of pneumonia which has not been previously reported as occurring in children.

Conclusions

These observations and experiments have established three facts:

1. That oil finds its way into the alveoli of the lung, not only when directly introduced into the trachea but also at times when administered in sufficient quantity in the nose and throat.

2. Oil when present in the lung is actively phagocyted by endothelial cells which are present in sufficient numbers to dispose of all the oil present and produce consolidation of the lung.

3. Argyrol, when given by mouth or introduced into the trachea, does not lead to any reaction on the part of the endothelial phagocytes in the lung.

The writer desires to express his thanks to Dr. Rittinger who assisted in carrying out the animal experiments; also to Professor Klotz, Dr. Magner and Dr. W. L. Robinson for helpful suggestions.

References

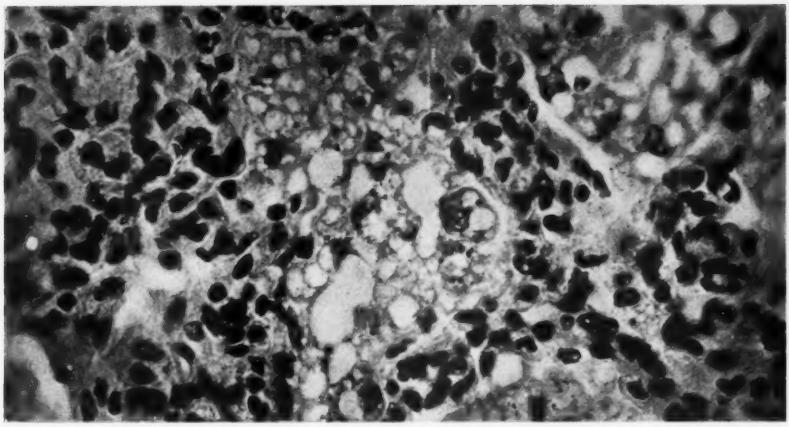
1. Permar. Jour. Med. Res., 1920, xlii, 9, 147.
2. Gilbert, A., and Jomier, J. Compt. rend. Soc. Biol., 1905, lix, 87.
3. Gravel, F. Compt. rend. Soc. Biol., 1919, lxxxii, 1329.

Description of Plate LXV

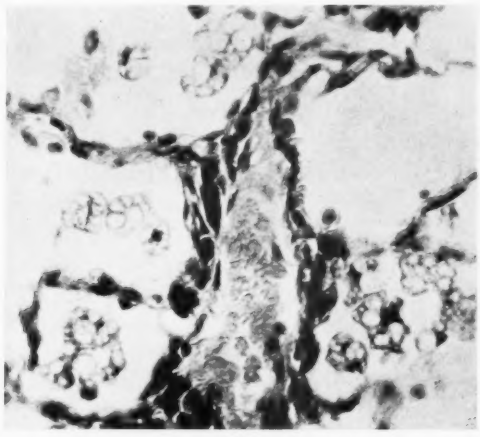
Fig. 1. This is a high power photograph showing oil phagocytes filling the alveoli. The outlines of many of the cells are indistinct but one large cell in the center of the field is clearly outlined and shows a rounded nucleus and a single nucleolus. This one contains two large droplets of oil. A few polymorphonuclear leucocytes are recognizable in the alveolar walls.

Fig. 2. Shows a number of oil phagocytes in the alveoli. They are not mixed with other cells and there is very little reaction in the alveolar walls.

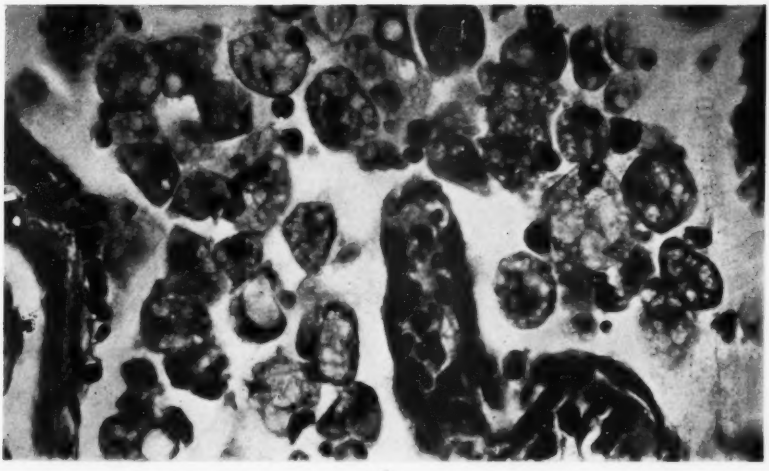
Fig. 3. Shows many large typical oil phagocytes intermingled with red blood cells.



1



2



3

Laughlen Pneumonia following injection of oil

THE PATHOLOGY OF DIABETES, WITH SPECIAL REFERENCE TO PANCREATIC REGENERATION*

SHIELDS WARREN, M.D., AND HOWARD F. ROOT, M.D.

(From the Pathological Laboratory, Boston City Hospital, and the Diabetic Clinic, New England Deaconess Hospital)

But little opportunity has been afforded for the pathological study of cases of diabetes treated by insulin and for determination of what effects, if any, this treatment has had upon the lesions. Twenty-six autopsied cases of diabetes, the disease process varying in duration from two months to thirty years, form the basis of this report. These cases were under the care of Dr. E. P. Joslin, and the histories and clinical findings are unusually complete, with only one or two exceptions. Of these twenty-six cases, seventeen were treated with insulin over periods ranging from ten hours to thirteen months.

In order to present briefly the salient facts in the pathology of these cases, they have been combined in Table 1. Although statements as to the exact time of onset of diabetes are somewhat uncertain, it has seemed best to arrange the cases in order of increasing duration. One case, where the patient had been under observation twenty-six days, but had undoubtedly been suffering from diabetes much longer, has been placed at the end of the table.

Pancreas. Though the weight or estimated size of the pancreas is given in most cases, it is of little value in estimating the amount of pancreatic substance owing to the great variation in the connective tissue and fat, chiefly interlobular, present in the organ. Thus of two pancreases, both of which contained but little pancreatic tissue, one weighed 30 gms. and one 240 gms. This variation is not altogether dependent on the weight of the individual from which the organ came, as both women weighed over 200 pounds.

No attempt has been made to count the number of islands, owing to the inaccuracy of any feasible method. However, in six cases the islands appeared to be distinctly less numerous than in normal pancreases. No one distinctive lesion of the islands has been encountered in this series, as is to be expected in light of the work of Allen,[1]

* Received for publication May 11, 1925.

TABLE 1

Number	Age	Duration of disease	Pancreas			Heart			Liver glycogen	Kidney		Aorta	Cause of death
			Wt. gms.	Islands	Acinar sclerosis	Wt.	Coronary arteries	Myocardium		Glycogen	Vascular disease		
4289	29.5	1 m. 27 d.	30	Lymphocytes	o	365	o	o	o	o	o	?	Septicemia
3592	64	6 m.+	116	H++	+	433	o	o	+	+	+	+	Multiple abscesses
2559	55	7 m.	?	H+++	o	?	S++	o	+	+	o	+++	Duodenal ulcer
3240	15.3	8 m.	Small	Few o	o	?	o	o	o	o	o	o	Coma
2720	19.2	11 m.	Small	Lymphocytes	o	?	o	o	+	+	o	o	Perinephric abscess
2446	36.9	1 yr. 1 m.	50	o	o	280	o	o	+	+	o	+++	Coma and pneumonia
1870	15	1 yr. 7 m.	Small	Lymphocytes	o	?	o	o	+	+	o	o	Coma
1907	24	1 yr. 10 m.+	Small	H++	++	?	o	o	?	+	o	o	Inanition
3210	65	1 yr. 10 m.+	Small	H+ few	++	250	S+++	S++	o	o	++	+++	Angina pectoris
2463	32.4	2 yr. 8 m.	Small	o	++	?	o	Hydrops	+	+	o	++	Abscesses of liver
4142	47.6	3 yr. 3 m.	80	H+++	+	290	o	o	+	+	++	+	Perinephric abscess
2988	56.3	4 yr.	Normal	H++	++	325	o	S+	o	+	+++	+	Pericarditis
1305	16	5 yr. 6 m.	Small	S+	++	?	o	S+	o	?	?	+	Coma
2479	69	9 yr.	Normal	o	++	325	S++	S++	+	+	++	+++	Chronic myocarditis
3679	46	9 yr. 6 m.	Small	Few S+++	+++	320	o	Hydrops S+	+	+	+	++	Appendicitis
896	69.2	10 yr. 8 m.	Cancer	H++	Cancer	330	S+++	S+++	+	+	+	++	Cancer of pancreas
3798	64	11 yr.	180	Few S+	++	315	S+++	S++	o	o	++	++	Gangrene and septicemia
705	66	14 yr.	?	H++	++	520	S+++	S+++	+	+	++	+++	Angina pectoris
1419	53.3	14 yr. 7 m.	240	S+H++	+	360	o	o	+	+	++	++	Appendicitis
263	48.7	14 yr. 9 m.	70	Few H+++	+	340	S+	S+++	o	o	++	++	Cardio-renal
870	53.2	16 yr. 11 m.	Normal	H+++		350	S+++	Aneurysm S+++	o	+	++	+++	Coronary thrombosis
3176	58.5	17 yr. 4 m.	Normal	S+++	+	?	o	S+	+	+	+	+	Phthisis
127	62	25 yr.	40	Few o	++	335	S+++	++	+	+	+	++	Coronary thrombosis
3242	52	25 yr.	?	H+++	++	?	+	o	o	o	o	?	Septicemia
1924*	53.9	30 yr. 2 m.	?	H++	+++	?	?	?	+	+	o	?	Gangrene and septicemia
3468	61	26 d. +	?	S+	++	610	S+++	S+++	o	+	++	+++	Gangrene and septicemia

* Restricted autopsy H = Hyalin S = Sclerosis ? = No data o = No pathology

Opie,[2] and Cecil.[3] Thirteen of the pancreases showed varying degrees of hyalinization of the islands. Only one of the thirteen patients whose islands showed hyalin change was under forty-five years old. Five showed more or less sclerosis of the islands, in three there was some lymphocytic infiltration about scattered islands, and there was no demonstrable pathology in the islands of five. The islands of three showed the type of enlargement described by Cecil [3] as adenomatous. In the few instances in which we found changes in the islands suggesting hydropic degeneration, post-mortem change could not be ruled out. The most likely case, No. 1870, only fifteen years old, died in coma after diabetes of short duration and was autopsied within an hour. No hydropic degeneration was apparent.

Through the kindness of Dr. Mallory we have been able to examine a slide of a pancreas sent to him by Dr. Willard S. Hastings from a case of fulminating diabetes. In this section every island shows extreme hydropic degeneration.

One outstanding feature of the various pathological changes is the wide variation in the condition of the islands in the same pancreases, and even in the same section. In one case of fourteen years' duration, No. 263, every stage is present from apparently normal islands to masses of hyalin imbedded in the stroma. We have found no pancreas in this series, no matter how severe the disease process or how marked the changes in the islands, in which a greater or less number of apparently normal islands could not be found. Moreover, a large number of pancreases from cases of diabetes other than those included in this series each showed at least a few apparently normal islands. Owing to the type of fixation of some of the tissues, Bensley's neutral gentian stain for the granules of island cells could be used only in a few cases. Through the courtesy of Mr. W. Bowie of Toronto we have been able to use his modification of Bensley's stain for the island cell granules in a number of cases where otherwise specific granule stains would have been impossible.

As regards the acinar tissue of the pancreas, varying degrees of sclerosis are present in some cases, independent of pathology in the islands. It is worthy of note that the acinar sclerosis is not apparent in any case much under two years' duration, even though the islands may show considerable change. In one case, No. 3592, which shows slight acinar sclerosis, the duration as given is six months, but an

accurate history could not be obtained and the disease probably had lasted for a much longer time. In another case, No. 896, most of the pancreas was replaced by a carcinoma and practically no acinar tissue was present. Islands persisted in the midst of the tumor and were very numerous in the tail, which had not been invaded by the tumor.

So far as we can determine there is no difference between the pancreases of those cases given insulin and those under dietary treatment alone, although we have the impression that in No. 263, treated with insulin for 13 months, there are more apparently normal islands than would ordinarily be the case.

There is no distinctive lesion in the young, uncomplicated cases of diabetes, as might be expected if there were one definite causal agent giving rise to the disease. Hyalinization of the islands occurs usually in those persons beyond middle age.

Heart. From this series, and from our experience with elderly diabetics in general, we feel that damage to the myocardium, chiefly through disease of the coronary arteries, is much more common in diabetics than in similar age groups from the general population. Of our seventeen patients over 40 years of age, three showed extensive healed infarcts in the wall of the left ventricle, one had an aneurysm of the wall of the left ventricle, five showed extreme sclerosis of myocardium (including two of the cases of infarct mentioned above), three showed moderate sclerosis (including the other case of infarct), four showed slight sclerosis and five showed none. The coronary arteries were correspondingly affected in these patients. In two cases there was occlusion of a main branch of the coronary artery, in one the left branch of the coronary artery was practically occluded, in seven there was marked coronary sclerosis, moderate sclerosis in two, slight in two, and no evidence of coronary disease in six. In two of the negative cases a single slide of the heart wall was the only tissue available.

Arteriosclerosis of the aorta generally ran fairly parallel with that of the coronary arteries, though in a few cases the aortic changes are either more or less marked than those in the coronaries. In one sixteen-year-old boy, No. 1305, who had very high blood fat, there were atheromatous plaques in the aorta and slight sclerosis of the myocardium.

We hope to make a detailed study of the heart and vessels in these and other diabetics, to be reported in a subsequent paper.

Liver. The only change characteristic of diabetes is the presence of glycogen within the nuclei of liver cells, and that is by no means pathognomonic of the condition. The liver cell nuclei show this deposit in fifteen of the cases.

The frequency of occurrence of gallstones in these cases is of interest, and is too great to be merely coincidence. Six cases, four of them males, totalling 30 per cent of those over thirty years old, showed gallstones at autopsy, and another case, also a male, had an obliterated gall bladder with numerous adhesions.

Kidneys. In the older cases there is a somewhat greater incidence of moderate chronic vascular nephritis than would be expected in a

TABLE 2

Insulin treated					Not insulin treated		
No.	Amount of insulin		Glycogen in liver	Glycogen in kidney	No.	Glycogen in liver	Glycogen in kidney
	Time	Units daily					
127	3 mos.	4–15	+	+	870	+	+
263	13 mos.	10–30	o	o	1870	+	+
705	6 days	2–6	+	+	1907	–	+
1305	11 weeks	2–16	o	–	1924	+	+
2446	1 day	100	+	+	2463	+	+
3176	8 days	15	+	+	2479	+	+
3240	10 hours	60	o	o	2559	+	+
3242	6 days	12–90	o	o	2720	+	+
3468	12 days	7–10	o	o	3210	o	o
3592	15 days	12–85	+	+			
3798	10 days	15–30	o	o			
4142	4 days	12–15	+	+			
3679	6 weeks	5–30	+	+			
2988	2 weeks	.....	o	+			
1419	1 day	50	+	+			
4289	22 days	5–75	o	o			
896	5 mos.	15–100	+	+			

similar age group of non-diabetics, 78 per cent as against 69 per cent in a series of 100 autopsies, done in this laboratory, of non-diabetic patients over forty-five years old. This corresponds well with the amount of vascular disease. In six cases we could find no evidence of glycogen in the epithelial cells of Henle's loops.

Glycogen. In Table 2 is shown the occurrence of glycogen in the nuclei of liver cells and in the epithelial cells of Henle's loops.

Forty per cent of the insulin-treated cases show no glycogen, while it is absent in only 11.1 per cent of the cases under dietary treatment. This last percentage is rendered unduly high by the small number in the series; it is quite remarkable to find no trace of glycogen in Henle's loops in diabetics who have not had insulin treatment.

Complications

Gangrene. Seven of these cases, summarized in Table 3, developed gangrene. There appears to be a definite relation between gangrene and obesity.

These eight cases average 44 per cent overweight, as against 26 per cent for the cases which did not develop gangrene.

Acidosis. In all except one of the ten cases which had severe acidosis, but not coma, the condition was brought on by an acute

Table 3

No.	Gangrene		Arteriosclerosis	Maximum weight above standard %	Age
	Location	Duration			
127 [1]	Left leg	20 days	Marked	55	62
1924	Right foot	4 days	———	66	54
2479	Left foot	14 weeks	Marked	40	69
3210	Left foot	2 weeks	Marked	11	65
3468	Right foot	10 days	Marked	46	61
3592	Right foot	15 days	Moderate	39	64
3798	Right foot	6 weeks	———	52	64

[1] Embolic in origin.

infection. That one exception was a young man of 24 whose diabetes was very severe. There is no apparent correlation between the condition and demonstrable pathology.

Coma. Of the six cases that died either in coma or within one month after recovery from coma, one omitted his insulin and had an infected hand, two broke diet, one developed pneumonia and another acute pericarditis, and one suffered from acute hyperthyroidism. Here again, as in acidosis, there is no characteristic pathological change. Four of the deaths occurred in those whose diabetes had lasted less than one year and seven months. None of these four

cases had demonstrable pancreatic pathology which could be clearly related to diabetes.

Out of 33 cases of coma reported by Joslin,[4] 17 occurred during the first year of the disease. That the first year of the disease is really the danger zone has long been apparent clinically [5] and generally explained as due to the patient's ignorance of means for its prevention or even to his ignorance of the existence of the disease. However, another factor must be considered; the regenerative power of the pancreas may be overwhelmed by the unknown toxic process at a time when the body metabolism is on a plane not yet adjusted to the disease. Especially in the young is this true for in them the metabolic requirements of growth add to the strain.

Obesity. There is a striking degree of obesity in most of these cases, particularly the older ones. The patients at their maximum averaged 32 per cent overweight, and if the five under 25 years of

TABLE 4

Obesity in Relation to Heart and Vascular Pathology

Case No.	Per Cent Overweight	Pathology
3468	46	+++
3210	11	+++
2479	40	++
896	46	+++
3798	52	+++
705	29	+++
263	35	+++
127	55	+++
870	16	+++

+++ = extreme coronary sclerosis {and / or} myocardial injury.

age are excluded, 37 per cent overweight. The weights given are for the most part those at onset of the disease. Unfortunately, the weight was not noted at autopsy in most cases. We feel, however, that the weight at death should be included in the autopsy data of all diabetic cases.

Duration. Several cases in this series were under observation for remarkably long periods. The lengths of time given are reckoned from the first definite diagnosis of the disease and so are minimal. The duration of the disease in seven is known for periods extending over fourteen to twenty-five years. One other, No. 1924, had been

refused life insurance repeatedly because of sugar in his urine thirty years before death. These clinically were relatively mild cases. In six of the eight cases the age at onset of the disease was under forty, and the average age at onset of all eight was thirty-six.

Of these eight cases, six showed a considerable degree of hyalinization of the islands of the pancreas, one showed marked sclerosis of the islands and extreme hyalin change of the arterioles of the pancreas, and the islands in another appeared normal, though seemingly decreased in number. In all the cases there was a greater or lesser degree of sclerosis of the acinar tissue.

The outstanding feature in all these cases is the presence of a few islands which show no demonstrable pathology, even though the majority may be seriously damaged.

Discussion

We have presented no direct evidence as to the etiology of diabetes, though obesity is very marked in some of these cases, especially in the older diabetics. The age at onset varies from ten to sixty years, and when above twenty years seems to have little effect on the duration of the disease so far as this series is concerned. The variation and inconstancy of the pancreatic lesions argue against an infectious origin for the disease, and the character of such lesions as may be present does not suggest the result of invasion by organisms. Indeed, one is tempted to wonder whether the changes in the islands may not be the result instead of the cause of diabetes. While it is true that in a few relatively early cases of diabetes we found lymphocytes about the islands, this can hardly be considered as evidence for an infectious origin of the lesion.

Whatever the cause may be, it seemingly acts over a long period of time, perhaps throughout the duration of the disease. The pathology which we find in the pancreas at autopsy rarely represents the initial damage to the organ, but rather the result of a long struggle between the regenerative activity of the pancreas and the degenerative changes caused by the diabetogenic factor. The pancreas is not a static organ like the brain or myocardium, unable to repair itself after injury.

For some reason this static conception of the pancreas has become firmly established in spite of clinical and anatomical evidence to the contrary, probably because the diabetic patient cannot be cured and

frequently goes steadily downhill in spite of treatment. We believe that this unfavorable course of the disease is not due to failure of the pancreas to regenerate, but to continued injurious action on the organ by the causal agent, eventually overcoming the regenerative efforts. In a subsequent paper we are presenting the clinical evidence given by this same series of patients.

Perhaps one reason for the skepticism as to pancreatic regeneration in diabetes is the lack of the classical evidence of such activity — cells in mitosis and cells in abnormal situations and relations with one another.

We have found evidences of the power of the pancreas and of the island tissue in particular to regenerate after acute injury. Thus in a pancreas from a non-diabetic patient dying of lobar pneumonia (A15–16) we found numerous mitotic figures in the island cells, as high as seven mitoses in a single island (Fig. 1). Mitotic figures can occasionally be found in the island cells of cases dying of diphtheria and of lobar pneumonia. At times necrotic cells are found. This injury and subsequent repair not only indicate the regenerative power of the pancreas, but perhaps explain the transient glycosuria occasionally encountered in acute infections.

This same transient injury to the islands may explain the severe drop in sugar tolerance noted in diabetic patients during acute infections. The rapid reëstablishment of the former level of sugar tolerance following recovery from the acute process may well represent the result of the rapid regeneration of the island cells.

Bensley [6] states that in experimental animals after duct ligation occasionally a pancreas will be found which differs from the normal only by smaller size and increased fibrous interstitial tissue. This is interpreted as due to regeneration following accidental reëstablishment of the connection between duct and bowel. Bensley believes that under appropriate conditions the capacity of the pancreas for regeneration is 100 per cent.

Boyd and Robinson [7] have reported a case of a nine-year-old diabetic boy, accidentally killed after six months of insulin treatment, whose pancreas showed definite evidences of regeneration and whose insulin requirement had steadily fallen.

In one of our cases, No. 896, whose diabetes had lasted for fourteen years and who had been under insulin treatment for five months, receiving 15 to 100 units daily with gradual decrease of the

insulin requirement, there is evidence of regeneration of the islands. No acinar tissue is present. The bulk of the pancreas is occupied by a carcinoma, from which the patient died, but the tail is not invaded by the tumor. Here the islands are closely packed. Some show a moderate degree of hyalinization, but most are entirely free from hyalin or other degenerative changes. Columns of cells extend out from the islands into the surrounding stroma, and in places entire low power fields are made up of island tissue. There are more islands than could be accounted for by their concentration by contraction of the stroma of the pancreas following destruction of the acinar tissue.

In any disease as insidious in onset and as chronic as diabetes, with pathological changes largely restricted to one portion of a single organ, one cannot expect any striking evidences of either destruction or regeneration. It is not unnatural that the conception of the diabetic pancreas as an inert organ, passively submitting to gradual destruction, has become firmly established.

We may assume that the lesions in diabetes are not infectious but toxic in origin. Their course is extremely chronic, and consequently the attempts at regeneration are slow. Mitotic figures would hardly be expected under the circumstances.

Practically all toxic lesions of the same age in the same organ resemble one another, as in toxic myocarditis or central necrosis of the liver. But in the islands of the pancreas showing either hyalinization or sclerosis, practically every stage from masses of hyalin or dense connective tissue imbedded in the stroma to apparently normal islands can be found. It is difficult to conceive a toxic substance of very chronic action or a long-continued functional strain totally destroying one island and completely sparing the next. Much more logical is the assumption that we are dealing with a gradual destruction of islands, a formation of new islands to replace them, exposure of these to the toxic substance with consequent pathological change and still more islands formed to take their places. The apparently normal cells found represent those most recently formed. Eventually the destructive process wears down the regenerative powers of the organ and the end comes.

Hemochromatosis, often known as bronzed diabetes, gives us an excellent opportunity to test this assumption. In this disease we are dealing with a known injurious agent, hemofuscin. This is a break-

down product derived from hemoglobin and is deposited in various cells of the body, where it very slowly changes to hemosiderin. Eventually the accumulated pigment causes necrosis of the cell containing it. The liver is the first site of deposit, but as its cells become filled, the pigment overflows, one might say, to other organs. The pancreas is one of these.

In those cases of hemochromatosis where the pancreas has become seriously involved before death, diabetes occurs. If the pathology referable to the pigment cirrhosis of other organs be disregarded, this diabetes differs in no whit from diabetes mellitus, except that the course is more rapid.

Here then we have an ideal means of studying diabetes, with a known etiology and a fairly rapid course.

We have had the opportunity of studying several cases of bronzed diabetes in this laboratory. We find the same variation in involvement of the islands as we have mentioned in our cases of diabetes mellitus, ranging from the remains of islands represented by clusters of pigment-loaded endothelial leucocytes and fibroblasts in the stroma to islands without pigment and apparently normal. The conclusion is inevitable that new islands are being formed to take the place of those destroyed by the pigment deposits. In further substantiation of this evidence, occasional mitotic figures (in Case A 17–8) can be found in the cells of the younger, pigment-free islands (Figs. 2, 3).

The pigment is not restricted to the island cells, but affects the acinar tissue as well. The same evidence of regeneration is offered by the acinar cells as by those of the islands.

The well-established evidence of destruction and regeneration of parenchymal cells in the liver offers a striking parallel to the changes in the pancreas in hemochromatosis. Just as in the liver the parenchymal cells show every stage from newly-formed pigment-free cells through those containing hemofuscin and those containing hemosiderin to necrotic cells, the same steps can be traced in the acinar and island cells of the pancreas.

If we substitute diabetes mellitus for hemochromatosis, hyalin formation in the islands for pigment deposit in the island cells, the analogy is complete.

There is no reason to doubt that the increased fibrous tissue noted in the pancreas in some cases of diabetes mellitus accumulates in the

same way as the fibrous tissue in cirrhosis of the liver. The parenchymal cells, sometimes of the islands, sometimes of the acinar tissue, or of both, are killed and disappear. Their stroma remains behind. The parenchymal cells regenerate and new stroma forms to support them. In this way the fibrous tissue gradually increases in amount. The increased fibrous tissue noted in the pancreas in some cases of diabetes is therefore not due to a simple proliferation of the interacinar and interlobular connective tissue. Probably in most cases there has been damage to and regeneration of the acinar tissue as well as the islands. There is some clinical evidence of disturbed external secretion of the pancreas in diabetic patients.[8]

In our series fibrosis of the pancreas is not found in those cases whose diabetes had existed less than two years. However, some of the cases of fairly long duration do not show any great increase in fibrous tissue.

Aside from the pathology of the pancreas, the changes in the blood vessels and myocardium are of interest. The frequency of severe myocardial damage and of sclerosis of the coronary arteries and the aorta is much greater than would be expected for similar age groups of non-diabetics. Of course our series is too small from which to draw any definite conclusions, but it does indicate an abnormal prevalence of vascular disease among diabetic patients. This may well be related to the abnormal fat metabolism and the striking tendency toward obesity.

In one sixteen-year-old boy (No. 1305) there were found at autopsy atheromatous plaques on the aorta. He had a high blood fat, and it seems quite possible that this is related to the arteriosclerosis. In addition, large numbers of lipoid-filled cells are present in the spleen. A similar case, a man of 22, reported by Smith,[9] showed slight atheromatous plaques in the aorta.

The frequency of chronic vascular nephritis in our series is also somewhat higher than is encountered in non-diabetic patients, reënforcing the other evidences of vascular disease.

So far as differences in pathological findings in those cases treated with insulin and those not treated with insulin are concerned, there is nothing startling. We have an impression that there are rather more normal appearing islands in the pancreases of insulin-treated cases than in those of cases under dietary treatment alone. Glycogen is much less frequently found in the liver cell nuclei and in the

cells of Henle's loops in insulin-treated cases, as might be expected with the improved utilization of carbohydrates.

Summary

1. The pathological findings in twenty-six cases of diabetes mellitus are presented. The duration of the disease in eight of these is known to be over fourteen years.

2. Thirteen cases show hyalinization of the islands of Langerhans. Only one of these cases was under forty-five years old. Five show varying degrees of sclerosis of the islands. In three more there is slight lymphocytic infiltration about scattered islands, and the islands in five appear normal, though decreased in number in two of these five.

3. Apparently normal islands are present in all pancreases examined, no matter how badly damaged the bulk of the islands may be. In two cases, insulin-treated over five months, there seem to be more islands of normal appearance than in cases under dietary treatment.

4. The character of the lesions in the islands of Langerhans suggests a toxic origin, and an injurious agent acting over a long period of time.

5. Occasionally in acute infections, such as lobar pneumonia and diphtheria, there is toxic injury of the island cells, and subsequent regeneration of the islands. This is probably the explanation of the transient glycosuria sometimes met with in the course of acute infections.

6. In hemochromatosis there is definite evidence of regeneration of both the acinar tissue and island tissue of the pancreas. The type of diabetes in hemochromatosis is the same as that in diabetes mellitus.

7. The increased fibrous tissue found in certain pancreases in diabetes is due to destruction of island or acinar cells or both, with persistence and condensation of their stroma. The parenchymal tissue regenerates and forms new stroma, with resulting increase in connective tissue.

8. The acinar tissue of the pancreas does not show evidence of increased fibrosis in cases of diabetes of short duration.

9. Insulin treatment decreases the frequency with which glycogen is found in liver cell nuclei and in the epithelium of Henle's loops.

10. The frequency of severe myocardial damage, due to coronary sclerosis, is high in diabetics over forty.

11. The patients developing gangrene averaged 44 per cent overweight, while the others averaged 26 per cent overweight.

12. The patients over twenty-five years of age in this series averaged 37 per cent overweight.

Conclusions

1. A new interpretation is offered of the pathology of the pancreas in diabetes mellitus.

2. The long-continued action of an injurious agent (or possibly excessive functional activity) causes a gradual destruction of island, and at times of acinar cells. New cells are formed to take the place of those destroyed, only to be exposed to the injurious influence with consequent pathological change. Their injury is followed by the production of still more new cells.

3. Eventually the destructive process wears down the regenerative powers of the pancreas, thus explaining the unfavorable course of the disease.

4. In pneumonia and other infectious diseases, the pancreas readily regenerates after acute injury.

5. The disturbed carbohydrate metabolism giving rise to abnormal fat or protein metabolites may be the cause of the high incidence of vascular disease in diabetic patients.

REFERENCES

1. Allen, F. M. Glycosuria and Diabetes, Boston, 1913.
2. Opie, E. L. Diseases of the Pancreas, Philadelphia, 1910.
3. Cecil, R. L. Jour. Exp. Med., 1909, xi, 266.
4. Joslin, E. P., Root, H. F., and White, P. Med. Clin. N. Amer., 1925, viii.
5. Joslin, E. P. Treatment of Diabetes Mellitus, Philadelphia, 1923, pp. 159, 317.
6. Bensley, R. R. Am. Jour. Path., 1925, i, 143.
7. Boyd, G. L., and Robinson, W. L. Am. Jour. Path., 1925, i, 135.
8. Jones, C. M., Castle, W. B., Mulholland, H. B., and Bailey, Francis. Arch. Int. Med., 1925, xxxv, 315.
9. Smith, M. G. Bull. Johns Hopkins Hosp., 1925, xxxvi, 203.

DESCRIPTION OF PLATE LXVI

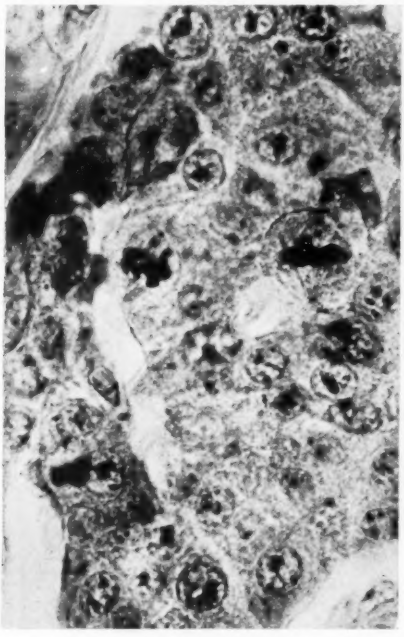
Fig. 1. Island of Langerhans from case dying of lobar pneumonia, showing three mitoses. x 1000.

Fig. 2. Island of Langerhans from case dying of hemochromatosis, showing pigment-free island and two mitotic figures. x 1000.

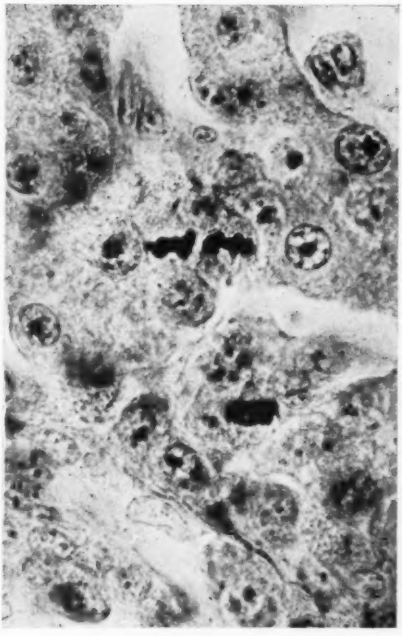
Fig. 3. Island of Langerhans from case dying of hemochromatosis, showing one mitotic figure, and small amount of pigment. x 1000.

Fig. 4. Island of Langerhans from case dying of hemochromatosis, showing one mitotic figure.

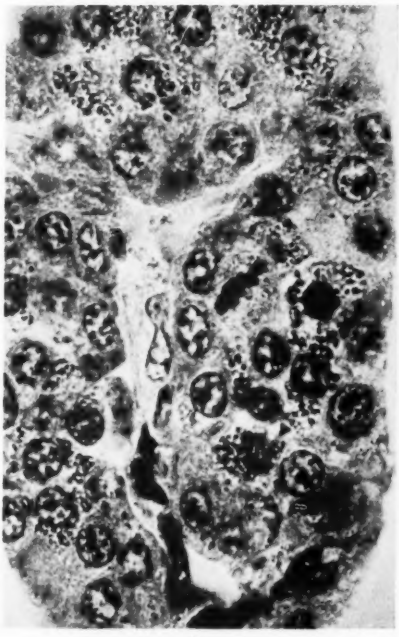
Photomicrographs by Dr. F. B. Mallory.



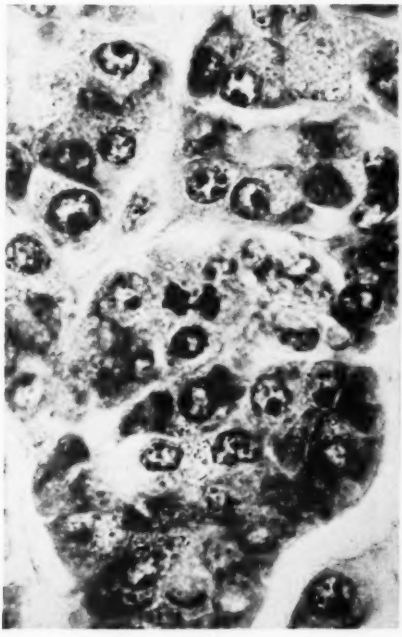
1



2



3



4

Warren and Root

The pathology of diabetes

THE OCCURRENCE OF RETICULUM IN TUMORS*

N. C. Foot, M.D., and H. A. Day, M.D.

(From the Department of Pathology, University of Cincinnati College of Medicine, and Cincinnati General Hospital)

Introduction. Since von Kupffer discovered the "Gitterfasern" of the liver in 1876, and Mall (1891, 1896) first distinguished the reticular connective tissue fibers from the collagenous fibers, reticulum has been the subject of considerable investigation; its occurrence in various organs under conditions of health and disease, its relationship to collagenous connective tissue, and its histogenesis (which is still in doubt) have all been studied. The reader may find an extensive bibliography in Russakoff's (1908) article on the pulmonary reticulum. At the time he wrote, the introduction of the Bielschowsky-Maresch silver impregnation technic had given impetus to the investigation of reticulum; Kon (1908) had studied it in the liver, Rössle and Yoshida (1909) in the lymphnodes, while Russakoff examined sections of liver, kidney, pancreas, and lymphnodes as well as lung. Neuber (1912) investigated the cardiac reticulum in health and disease, and more recently Corner (1920) and Miller (1923) have attacked the problem as it applies to the capillaries and the lung.

A survey of this work would lead the reader to conclude that reticulum is the product of cellular activity, that it may be connected with the blood-vessels on the one hand, or serve as a supporting, reticulated membrane for epithelium on the other. It forms practically the sole supporting tissue in lymphnodes. That it is the product of endothelial cells and, occasionally, fibroblasts, seems to be the general belief; some authorities claim the reticuloendothelium, some the vascular endothelium as well, as its progenitor.

One may readily study the distribution of the reticulum in various organs by means of silver impregnations according to Bielschowsky's technic, so that further preliminary discussion of this subject is unnecessary; let it suffice that it is very widely distributed and its

* Received for publication June 1, 1925.

importance is often overlooked. For example, its widespread occurrence in epithelial organs, where it forms a dense basement membrane, is insufficiently recognized. That it may with time, and under rather uncertain conditions, become transformed into collagenous fibrous tissue is becoming more universally believed. Russakoff, Rössle and Yoshida, and Miller have all stressed this point. That it is the product of cellular activity on the part of endothelial derivatives is, however, becoming a debatable question. Miller has pointed out the close relationship between newly-formed reticulin fibers and neighboring, preëxisting reticulum; Baitsell (1915, 1916) has advanced anew the theory of purely extracellular origin; and one of us (Foot, 1925) has recently stated his belief that we must look elsewhere than to cells for the origin of reticulin fibrils, basing this view on observations on the development of reticulum in experimental tubercles.

As nothing appears to have been done in connection with the distribution and occurrence of reticulum in tumors, a hundred or more have been collected in the past three years' accumulation of laboratory material of this department, and these have been studied with special reference to their reticulum content.

Technic

In most cases Zenker-fixed material is available, but where it is not, formalin-fixed tissue has been used. Several serial sections were cut from each paraffin block, three being stained with the routine Harris' hematoxylin-eosin stain and two by a modification of the Bielschowsky-Maresch technic devised by one of us last year. (Foot, 1924.)

Briefly summarized, the steps are as follows. Sections about five microns in thickness are cut in paraffin. Zenker fixation is superior to formalin, but the technic is the same in either case. After removing the paraffin, treat five minutes with a weak alcoholic solution of iodine, removing this by a brief immersion in 5 per cent "hypo." Wash and treat for five minutes with 0.25 per cent potassium permanganate in water. Wash and place the sections in 5 per cent aqueous oxalic acid. Wash in distilled water and impregnate for forty-eight hours with a 2 per cent solution of silver nitrate in distilled water. Wash in distilled water and treat with Bielschowsky's silver-ammonium oxide solution for one half-hour. Rinse in distilled

water and leave in 5 per cent formalin (2 per cent formaldehyde) for another half-hour and then, after washing in tap-water, tone the sections for one hour in a one per cent aqueous solution of gold chloride, after which a two-minute immersion in 5 per cent "hypo" will remove any superfluity of the silver. The sections may be mounted at this point, but it is better to counterstain for the usual time in Harris' hematoxylin, wash, and stain for forty-five seconds in Van Gieson's picric-acid-acid-fuchsin. Run the sections immediately through ascending percentages of alcohol into xylol and mount in balsam, avoiding water as it decolorizes the fuchsin. After the silver impregnation the sections should be practically colorless, turning brownish-black in the formalin and fading to gray in the gold bath. The "hypo" turns them old-rose and gray. The finished, counterstained sections show the reticulum in sharp black lines, the collagen in vermilion to crimson. The nuclei are brownish, the cytoplasm and muscle substance yellow. The silver impregnation is performed in subdued daylight, not in the dark.

Reticulum Fibers in Tumors

Epithelial Tumors

Surface epithelium. The epidermoid carcinoma is of paramount interest here, the benign epithelial tumors of this type conforming closely to normal histology. In these carcinomata there is a varying amount of reticulum; plentiful in the corium, it forms a network of delicate fibrils about the advancing columns of tumor cells which dip down from the epidermis. Generally speaking, if the tumor be of rapid growth reticulum is plentiful, if slowly growing more collagen than reticulum develops. Usually the latter forms a membrane-like plexus at the base of the epithelial plugs, occasionally it penetrates them superficially. A metastasis from such a tumor is shown in Fig. 1. In the basal cell, or hair-matrix carcinoma the reticulum forms a delicate basket-work around the cell masses, with radiating fibrils that are continuous with those of the corium.

Glandular epithelium. The common adenoma usually shows more collagen than reticulum in its stroma, the various types of fibroadenoma following this rule, but a narrow zone of reticulum is commonly found about the base of the acini, outlining them in black. In a rapidly growing papillary adenoma the stroma is composed al-

most entirely of reticulin fibrils, to the comparative exclusion of collagen. If the tumor acini are closely compressed one observes reticulin fibrils between them; lying almost, if not quite isolated and free from any great admixture of mesenchymal cells. Capillaries carry a sheath of reticulum, but frequently there are but few vessels in this stroma and the reticulum appears to have been produced independently of cellular agency.

The various types of adenocarcinoma and medullary carcinoma all follow the description just given; the cell-masses in the medullary type are usually quite free from penetration by the reticulum, but very rapidly growing tumors, with a tendency to cell dissociation, may show a good deal. Fig. 2 shows a typical adenocarcinoma. If the tumors have metastasized to lymphoid tissue the lymphoid reticulum will, naturally, be found intimately intermingled with their cells. In the colloid type, those cells which remain at the center of the mucoid masses may have small, woolly plexuses of what seems to be reticulum about them, but the production of fibrils resembling reticulin is quite common in necrotic areas in epithelial tumors and it is doubtful if such fibrils constitute true reticulum. Scirrhous carcinoma, as one might expect from its slow, sclerotic growth, shows large amounts of collagen in its stroma and a very variable quantity of reticulum, which is usually scanty. The sarcomatoid type of pancreatic carcinoma, shown in Fig. 3, presents a moderate amount of reticulum in its stroma and there is some penetration of this tissue into the more or less dissociated groups of tumor cells, which is also true of the similar type of carcinoma of the thyroid. The difference in the distribution of reticulum in these tumors and that in lymphosarcomata is, however, so striking as to render a diagnosis comparatively simple in cases that would be quite obscure when stained in the usual way.

Hypernephroma, or adrenal carcinoma, shows a very small amount of reticulum which does not penetrate into the alveolar cell masses. Although this tumor is found in the kidney, an organ rich in reticulum, there is practically none in the alveoli which push the preëxisting fibers aside.

The tumor section photographed for Fig. 4 was kindly sent to us from the Albany Hospital, by Dr. Victor Jacobson, and is worthy of special mention. It is from a medullary carcinoma of the uterus, which differs in no way from any other such carcinoma excepting

that its stroma shows an extraordinary anaplasia to what appears to be a true fibrosarcoma. Thus two malignant tumors are intimately combined in one section. The carcinoma is practically free from reticulum while its stroma shows an abundance of straight, coarse reticulin fibrils exactly analogous to those of the fibrosarcomata to be discussed presently.

Endothelial Tumors

Vascular. Cavernous hemangiomata show a reticulum about their vessels and sinuses quite similar to that normally seen surrounding capillaries. A malignant perithelioma appears to produce very little reticulum, as this is found only in the immediate proximity of the vessels. As we could secure only one specimen, this negative finding should be confirmed.

Reticuloendothelial. Several types of malignant endothelioma were examined, some showing comparatively little reticulum, some a great deal. Of the latter group, the primary lymphnode endothelioma, so closely resembling medullary carcinoma, is a good example. Where the carcinoma shows little reticulum in its alveoli, the fibers usually stopping short at their margin, or simply traversing them in straight, unbranching lines, the endothelioma exhibits an abundant, branching plexus of curving reticulin fibrils intimately associated with the cells of the tumor within its alveoli. The fibrils are distinctly plexiform and anastomotic in this case, which affords a good criterion for the diagnosis of doubtful alveolar tumors of the axillary lymphnodes in women, where primary endothelioma is readily mistaken for the more common metastatic alveolar carcinoma of the breast. We feel this to be the chief contribution of this paper. (Figs. 5 and 6.)

Another type of endothelioma, supposedly originating in the reticuloendothelium of lymphnodes, is composed of polygonal, anastomosing cells, and shows an abundant reticulum intimately related to these cells and following their cytoplasmic processes very closely. A given fibril may, however, course over several cells without any interruption in its substance and the unbroken continuity of these fibrils with those of the neighboring normal tissue is always striking. This tumor (Fig. 7), was recently reported by one of us (Foot, 1924) in detail.

The endotheliomata arising in bone-marrow correspond in the

main with the loosely reticulated portions of the primary endothelioma of lymphnodes, insofar as their reticulum is concerned; but they contain somewhat less and the fibers are far more delicate. One case of diffusely disseminated cells, resembling endothelial phagocytes and occasionally grouped into tumor masses, shows marked reticulum production wherever the cells become so massed.

Dural endothelioma. This tumor (Fig. 8) probably produces more reticulum than any other tumor we have observed; there is a dense felting of reticulin fibrils in its matrix and they are intimately intermingled with an almost equally dense mass of collagen fibrils.

Mesothelioma

A tumor from the pleural cavity, connected with the lung, is composed of squamous cells that tend to keratinize and to form pearls. It resembles an epidermoid carcinoma in every respect, but there is little upon which to base this diagnosis if one considers that the tumor lay in and beneath the visceral pleura, not near large bronchi, and there was no history to suggest bronchiectasia. A similar growth is described by Beitzke, in Aschoff's "Pathologische Anatomie" (1923), as a "pleural cancer." We prefer the term "mesothelioma" and feel security in our diagnosis for the reasons just given. This tumor shows an abundant reticulum between its cell masses and a moderate amount of collagen; it is almost exactly like an epidermoid carcinoma in respect to its stroma. Its cell masses are entirely free from reticulin fibrils.

Fibroblastic Tumors

Fibroma. Both types of fibroma, the soft and hard, are chiefly collagenous; practically no reticulum is seen, save around the blood vessels. (Fig. 9.) A very mucoid fibroma of the external auditory canal, which recurred twice in a year, shows rather scanty collagenous tissue with wide, unstained spaces and an abundance of short, rather straight reticulin fibrils. (Fig. 10.) It is not a true myxoma, for there is no bluish hyaline-matrix, and it is not malignant. Here there appears to be a direct causal relationship between the presence of reticulum and rapidity of growth.

Keloid. Both the keloid and the fibrous granuloma show reticulum and collagen in about the same proportions as occur in normal corium; the total amount of these fibers, however, is greatly in-

creased. The keloid has rather more collagen than reticulum, however.

Giant cell tumors. These are not rich in reticulum and show only moderate amounts of collagen. The giant cells become deeply stippled with silver, as is the case with some endothelial cells and with nerve cells. The tumors are chiefly cellular.

Fibrosarcoma. Two types are recognizable: one producing almost nothing but reticular matrix (Fig. 11), and one an abundance of collagen (Fig. 12). The former is very similar to the reticulo-endothelioma with branching cells, already described. Apparent transitions between the reticular and collagenous types are seen, but the degree of anaplasia and the rate of growth will not entirely explain this difference — for one very anaplastic and rapidly growing fibrosarcoma of the lung shows little or no reticulum, but produces an abundant collagenous matrix (Fig. 13). Generally speaking, however, there is more reticulum in the rapidly growing sarcomata and more collagen in those of slower development.

It is interesting to compare the tumors in our series of fibrosarcoma as regards their reticulum content. One, a sarcoma of the scapula (Fig. 14) of rapid growth, shows an evenly distributed mesh of very fine, short, and curly reticulin fibrils, many of them beaded and interrupted. This indicates very young reticulum, judging from the literature and from personal observation on its formation in tubercles. Very little collagen is present. Then come a sarcoma of the lung, one of the kidney, and a third from the bladder, all of which show coarse, straight reticulin fibers and little collagen. At the other end of the scale we find a sarcoma of the lung and one of the knee, both of which show much collagen and little reticulum. From this one may infer that reticulum is laid down by the more embryonal type of rapidly growing tumor and that it may ultimately be changed into collagen. The very primitive fibrosarcomata and reticuloendotheliomata both revert to a common type, the mesenchymal. Hence their similarity and the comparative uselessness of attempting to classify them by adult-cell standards, or to distinguish them apart.

Other Connective Tissue Tumors

Lipoma. Tumors of this type produce more collagen than reticulum, as one would expect after a survey of normal fat.

Liposarcoma. In one case we find short, wavy, more or less beaded reticulin fibrils and little collagen. (Fig. 15.) In the others, which resemble Fleming's fat organ, there is abundant reticulum and but little collagen.

Leiomyoma. Here much collagen is produced; often more of this is seen than of muscular tissue and a large amount of reticulum closely invests the muscle fibers. Is this reticulum converted into the abundant collagen that gives the clinical name of "fibroid" to the tumor?

Leiomyosarcoma. This shows an abundance of both reticulum and collagen, the latter in the form of short fibers and not at all as profuse as in its benign prototype. Here again we see more reticulum in the more youthful form of tumor.

Rhabdomyosarcoma. One of these, from the endometrium of an old woman, was examined. It is seen to be composed of alveolar septa of collagen, and in the spaces thus formed is a very loosely made network of reticulin fibrils, sometimes short and curly, sometimes nearly straight and much longer, but always wavy in contour. In the smaller spaces between these fibrils lie the rhabdomyoblasts, usually quite isolated. An abundance of cells resembling fibroblasts are also present and the reticulum is more apt to adhere to these than to the muscle cells.

Melanosarcoma. Melanosarcomata are abundantly supplied with collagen, but a good deal of reticulum is found penetrating the alveolar cell masses and running among them almost as freely as in the primary endothelioma of lymphnodes.

Chondroma and chondrosarcoma. Benign chondroma shows practically no reticulum, while chondrosarcoma shows a great deal in its more immature portions, the production of chondromucin later obscuring it. Sometimes the reticulum persists in this matrix and its fibers may be seen through the homogeneous chondromucin.

Osteoma. Aside from the marrow spaces, no reticulum is seen in this form of tumor. We did not obtain an osteosarcoma and cannot report on its reticulum.

Lymphoid Tumors

Lymphoma and Lymphosarcoma. Here one finds practically no fibers other than reticulum, and this may be quite unassociated with any cells resembling reticuloendothelium; in one malignant lym-

phoma of the small celled type the reticulum lies quite free in a mass of dissociated microlymphocytes, and one can see no connection between it and any other type of cell. It appears to have been laid down by some process independent of cellular activity, or borrowed from the lymphoid tissue.

The commoner lymphosarcoma, with its larger type cell, exhibits an abundant reticulum that branches all through the tumor and is in unbroken continuity with that of the lymphnode. (Fig. 16.) Where the tumor penetrates the capsule and invades the surrounding tissue, it forms a new reticulum that is continuous with the older.

Hodgkins' granuloma. The amount of reticulum produced in the Hodgkins node varies a great deal; sometimes there is much, at others (Fig. 17) there is practically no new reticulum to be found.

Tumors of the Nervous System

Glioma. This shows no reticulum other than that of its vascular supply.

Neuroblastoma and Ganglioneuroma. The same is true in this case, which makes it possible to differentiate these neoplasms from other types which might be confused with them. Neuroblastoma often resembles lymphosarcoma in routine sections, but a reticulum stain will readily differentiate the two.

Amputation neuroma and neurofibroma. There is very little reticulum in these, and it is confined to the connective tissue of the epineurium and its prolongations. In these there is much collagenous tissue.

Pituitary struma. One chromophile struma was examined, from a case of well-marked acromegaly. This tumor is so dissociated in its structure that practically the only stroma present is seen in the immediate vicinity of its vessels, in the form of perivascular reticulum.

Mixed Tumors

Mixed tumor of parotid. Several of these were studied, all showing a variable amount of reticulum about the epithelial islands and ducts. Collagen varies, being most abundant in the more mixed, and therefore more mature varieties; with cartilage and heavy connective tissue septa. The more youthful, epithelial type of tumor shows practically no collagen and a very dense reticulum about its ducts and acini. (Fig. 18.)

Teratoma. Several of these, one a malignant embryoma with rather advanced differentiation, were examined. No rule can be laid down for such diversified tumors; if they contain those types of tissue normally rich in reticulum, the latter will naturally be present.

Summary

From what we have said, it is evident that reticulum is a common and widely distributed constituent of the stroma and matrix of tumors, the only marked exceptions being those of the nervous system. Some of the very fibrous tumors show so much collagen that the reticulum is overshadowed and may, indeed, have been replaced by this tissue. In general, there appears to be a definite connection between rapidity of growth and reticulum formation, for this tissue is more abundant in young and rapidly growing tumors than in those of more adult type and slower progress. It is indicated that this would be converted into collagen in time; that this is not an invariable rule is shown by the presence of much collagen in some rapidly growing sarcomata. That anaplasia is not necessarily connected with the production of reticulum is indicated by the coincidence of reticulum and good differentiation in a benign fibroma, several lipomata, and other mature tumors and by its absence in tumors showing marked anaplasia.

One point that cannot be stressed too emphatically is our failure to find any definite relationship between cellular structures and reticulum. Although it is usually near vessels and is undoubtedly more abundant in their vicinity, it often appears to be quite unassociated with cytoplasm. It is often as abundant in the proximity of epithelial cell masses as it is near endothelial or fibroblastic cells, although it is undeniably more abundant in the case of endothelial tumors. It seems that the conditions for its production are more favorable in these tumors but that nothing points directly to the cellular origin of reticulin fibrils. As in the case of tubercle reticulum, there appears to be a closer relationship between reticulum and preëxisting reticulum than there is between that tissue and any cells. It is evident that we must keep our minds unclouded by dogma and further test out the hypothesis of the intercellular, or extracellular, origin of reticulum. The production of these fibrils may prove to be more or less similar to that of fibrin, a process of precipitation and accretion, rather than one of intracellular differentiation.

Conclusions

1. Reticulum is a regular constituent of the stroma of most tumors, excepting those of the nervous system.

2. It is usually most abundant in tumors of rapid growth.

3. It is apparently converted into collagen in the more slowly growing neoplasms.

4. It does not show any constant relationship to cellular constituents of tumors, but seems to be laid down in continuity with preëxisting reticulum in the intercellular fluids or substances by a process independent of cytoplasmic differentiation and analogous to precipitation or crystallization.

5. Silver impregnation of tumor reticulum constitutes a valuable diagnostic method, especially in the case of tumors of endothelial, lymphoid, and nervous-tissue origin.

This work represents the examination of 135 tumors, of which: 23 were adenocarcinoma, 11 fibrosarcoma, 10 lymphosarcoma, 9 epidermoid carcinoma, 8 endothelioma of various types, 7 fibroma, 4 leiomyoma, 4 lipoma, 4 Hodgkins' nodes, and 4 melanosarcoma. The rest were represented by one or two examples, either because it was obvious that they were typical, or because of lack of material, as in the case of neuroblastoma, ganglioneuroma, and rhabdomyosarcoma.

REFERENCES

Baitsell, G. A. 1915. Jour. Exp. Med., xxi, 455.

——. 1915–16. Anat. Rec., x, 175.

——. 1916. Jour. Exp. Med., xxiii, 739.

Beitzke, H. 1923. Pathologische Anatomie, L. Aschoff, 6th ed., Jena, ii, 326.

Corner, W. G. 1920. Carnegie Institution of Washington, Contrib. to Embryology, ix, 85.

Foot, N. C. 1924. Jour. Lab. & Clin. Med., ix, 777.

——. 1924. Jour. Med. Res., xliv, 417.

——. 1925. Am. Jour. Path., i, 341.

Kon, Y. 1908. Arch. f. Entwicklungsmechanik d. Organ., xxv, 492.

Kupffer, C. von. 1876. Arch. f. mik. Anat., xii, 351.

Mall, F. P. 1891. Abhndl. d. math. phys. Classe, Kgl. sächs. Gesellsch. d. Wissensch., xvii, 299.

——. 1896. Johns Hopkins Hosp. Rep., i, 171.

Miller, W. S. 1923. Am. Rev. Tuberculosis, vii, 141.

Neuber, E. 1912. Beitr. z. path. Anat. u. z. allg. Path., liv, 350.

Rössle, R., and Yoshida, T. 1909. Beitr. z. path. Anat. u. z. allg. Path., xlv, 110.

Russakoff, A. 1908. Beitr. z. path. Anat. u. z. allg. Path., lxv, 476.

DESCRIPTION OF PLATES LXVII–LXXI

PLATE LXVII

Fig. 1. Metastasis from an epidermoid carcinoma of the penis. The epithelial alveoli are not invaded by reticulum, which merely outlines them.

Fig. 2. Medullary form of adenocarcinoma primary in stomach. Here there is more dissociation of the cells and consequently slight invasion of cell masses by reticulum.

Fig. 3. The sarcomatoid type of pancreatic carcinoma. There is comparatively little reticulum, which tends to invade cell aggregations. Cf. with lymphosarcoma photomicrograph.

Fig. 4. Medullary carcinoma of uterus, with sarcomatous stroma. Specimen of Dr. Jacobson's. Note the very heavy, coarse reticulum of the stroma, and compare with that of the preceding figures and with that of the fibrosarcomata.

PLATE LXVIII

Fig. 5. Primary endothelioma of lymphnode. Compare this reticulum, which traverses the cell masses in all directions, with that of Fig. 6 and note its extensive branching and its gently curving course.

Fig. 6. Medullary type of adenocarcinoma of breast for comparison with Fig 5. This shows the greatest amount of invasion of the epithelial cell masses by reticulum that we have observed. There is a much straighter, simpler reticulum in this tumor.

PLATE LXIX

Fig. 7. Malignant reticuloendothelioma. The reticulum is very intimately associated with the tumor cells in this case.

Fig. 8. Dural endothelioma. The coarsest, densest reticulum observed in any of our tumors; very few of these fibers are collagenous in nature.

Fig. 9. A fibroma molle of the vulva. The black fibers are reticulum, or partially impregnated collagen, the gray are collagenous.

Fig. 10. A soft, mucoid fibroma of external auditory meatus. No appreciable amounts of collagen, much fine reticulum.

PLATE LXX

Fig. 11. A fibrosarcoma of the lung. The reticulum is fairly coarse and straight, and collagen is negligible.

Fig. 12. A fibrosarcoma of the knee. There is much collagen, which photographs grayish, and rather sparse reticulum.

Fig. 13. A fibrosarcoma of lung with marked anaplasia, but abundant collagen and comparatively little reticulum.

Fig. 14. A very rapidly growing fibrosarcoma of the scapular region, with extremely young and delicate reticulum and little collagen.

PLATE LXXI

Fig. 15. A liposarcoma of the sacral region. This shows very little, young reticulum. Note the cytoplasmic network. Little collagen.

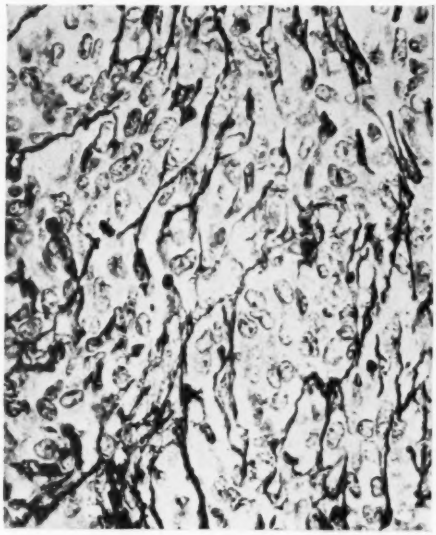
Fig. 16. Lymphosarcoma originating in the thymus. It is typical of the lymphosarcomata we have examined from other localities. Very abundant reticulum, often young and beaded, and usually unassociated with large cells. Cf. Fig. 3.

Fig. 17. A section of lymphnode in Hodgkins' disease. This case shows little reticulum, probably all of it from the original lymphoid reticulum.

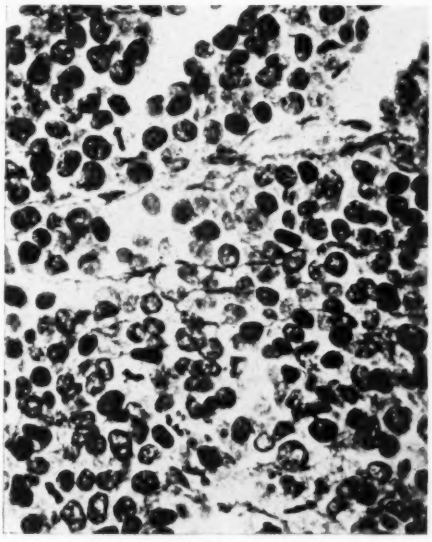
Fig. 18. A tumor from the parotid region, which is partly, if not entirely, epithelial in its make-up. It shows a very dense reticulum and well illustrates the relationship of epithelium to that tissue.

All these photomicrographs were taken at ×650 diameters, 4 mm. objective and ×15 ocular, with a short bellows (17 cm.).

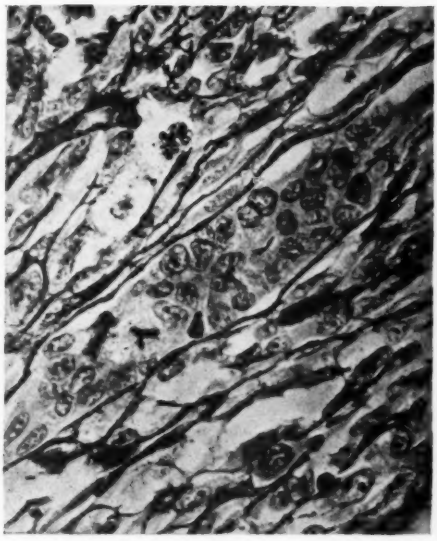
1



2



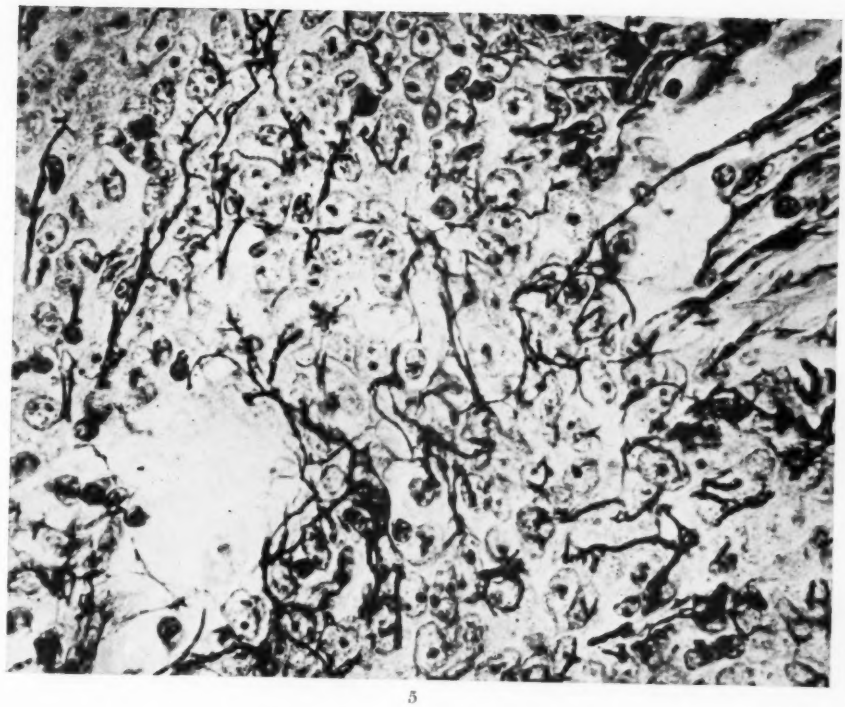
3



4

Foot and Day

Reticulum in Tumors

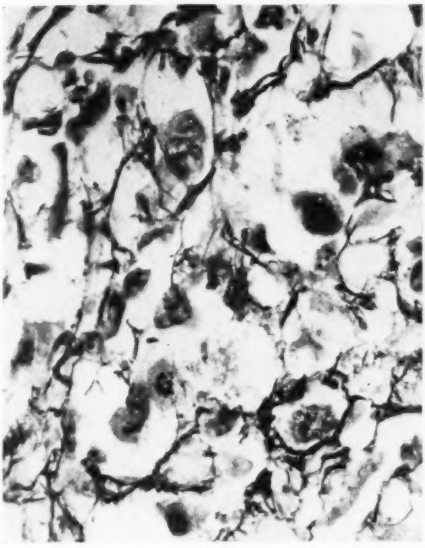


5

6

Foot and Day

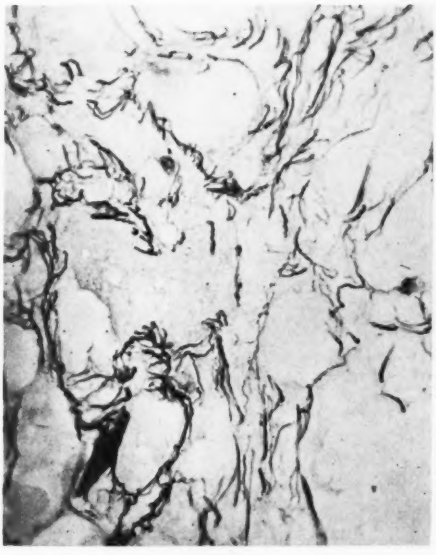
Reticulum in Tumors



7

8

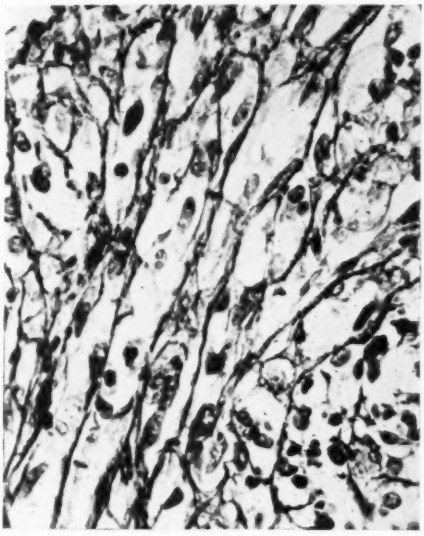
9



10

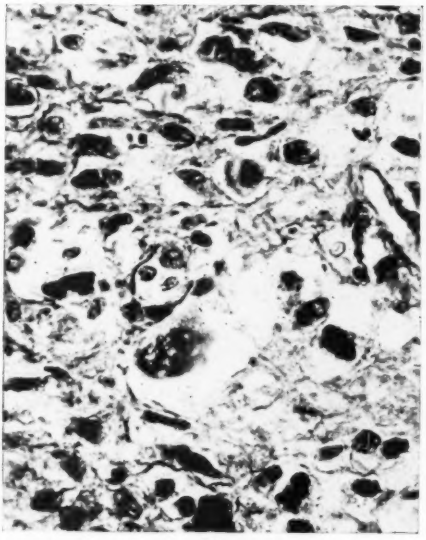
Foot and Day

Reticulum in Tumors



11

12

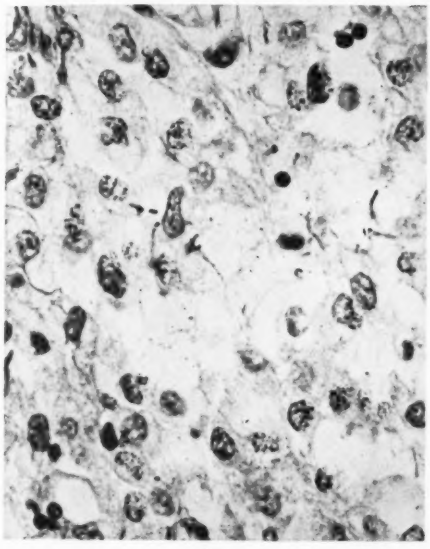


13

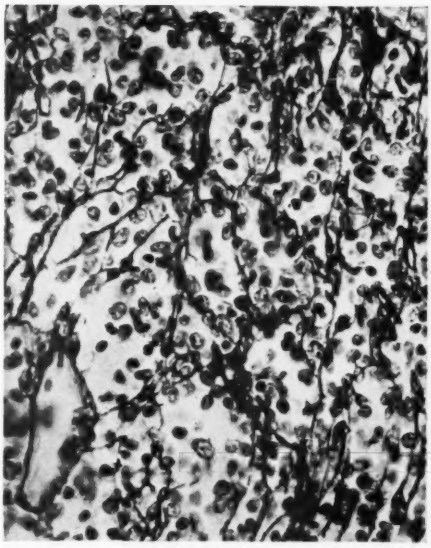
14

Foot and Day

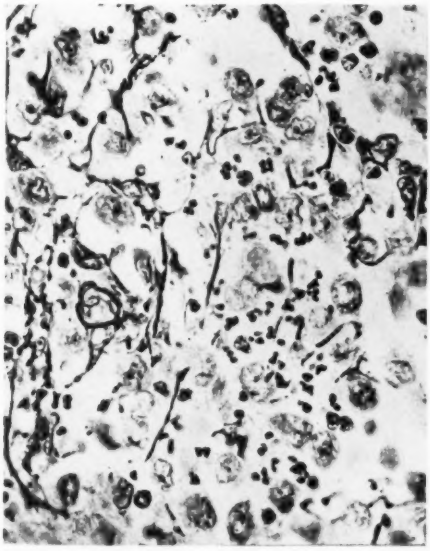
Reticulum in Tumors



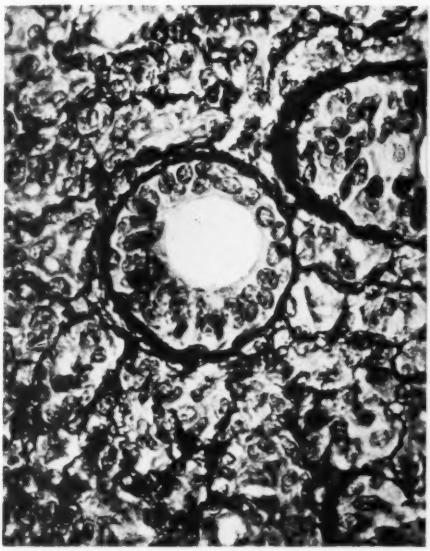
15



16



17



18

Foot and Day

Reticulum in Tumors